GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

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CORRIGENDA

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Page 88, line 12, for "6.9 to 51.1" read "6.9 to 55.1."
Page 121, line 9, for "inviability" read "viability."
Page 298, figure 2, the abscissa of the lower right graph should be reversed.

Lamarck

JEAN BAPTISTE-PIERRE ANTOINE DE MONET, CHEVALIER DE LAMARCK

THE YEAR 1944 marks the two-hundredth anniversary of the birth of one of the greatest and yet most tragic and long-neglected figures in the history of the biological sciences—LAMARCK. He was born on August 1, 1744, in a little village in Picardy, the youngest of eleven children of a noble but impecunious family. His male ancestors for several generations had been soldiers, and although his parents had destined him for the priesthood and had sent him for a short time to the Jesuit College at Amiens, he abandoned this course on the death of his father in 1760 and joined the French Army in Germany near the close of the seven year war. He was soon raised to the rank of lieutenant for gallantry in service and continued in garrison duties for five years but was compelled to abandon his military career because of an accident which required prolonged surgical treatment.

While in garrison at Monaco he had become interested in botany, and now at the age of 22 and with no other resources than a *pension alimentaire* of 400 francs a year, he resolved to devote himself to the study of natural science. He lived in a garret in Paris, earned a bare living as clerk in a bank, studied medicine for four years, and meanwhile collected plants in the vicinity of Paris. He met JEAN JACQUES ROUSSEAU, who was also interested in botany, and the two made botanical excursions together and no doubt discussed many scientific and philosophic topics.

Thereafter botany absorbed LAMARCK to such an extent that he abandoned medicine and devoted the next ten years to the study of the French flora. In 1778 he published his first major work, *Flore française*, in three volumes. With the aid of BUFFON it was published at the royal press at government expense, and it at once placed LAMARCK in the front rank of botanists. In 1779 he was appointed by the king to a vacant seat in the Academy of Sciences in preference to DESCEMET—a great and rare distinction for a young man of thirty-five. BUFFON procured for him a commission as Royal Botanist, charged with visiting foreign botanical gardens and museums, and in company with BUFFON'S son he visited principal scientific centers and scientists in Germany, Hungary, and Holland. He returned in 1782 with a considerable collection of natural history objects, but he had no salaried position until 1789, when he was appointed keeper of the herbarium at the *Jardin du Roi* at the small salary of 1,000 francs a year, and in 1790 even this was threatened with suppression by the *Assemblée Nationale*. LAMARCK, with a wife and six children, was in dire straits, and he made a strong plea for his continuance and drew up a plan for the development of a great *Museum d'Histoire Naturelle*. Such a museum was established in 1790, and continued by the National Convention in 1793, at which time the name of the *Jardin du Roi* was changed on LAMARCK'S suggestion to *Jardin des Plantes*.

LAMARCK was appointed one of the six professors at the Museum. The zoology of vertebrates was assigned to GEOFFROY ST. HILAIRE and all the rest of the animal kingdom to LAMARCK. Thus in his fiftieth year he turned from botany to invertebrate zoology and began a new career. Amidst all the disturbances of the Revolution and the years that followed he worked industriously at the Museum classifying the enormous collections, lecturing to classes, and publishing articles and books. The extraordinary breadth of his knowledge of plants and animals led to his being called the "Linné of France"; his unselfish and retiring disposition endeared him to his associates; but his philosophical and theoretical works brought upon him the condemnation and ridicule of CUVIER, the favorite of fashionable and learned circles in France, and his work was but little regarded in other countries. In 1818 he became totally blind, and for the remaining ten years of his life he carried on his work with the devoted aid of his oldest daughter, CORNELIE, without whose assistance his greatest work *Animaux sans Vertebres* in seven volumes would never have been completed. He died on December 28, 1829, and was buried in an unmarked and now unknown grave.

Revival of interest in LAMARCK is due largely to his theoretical work on evolution, or what he called "transformism," and dates from the publication of CHARLES DARWIN'S *Origin of Species* in 1859. In his earlier work LAMARCK apparently accepted the then current view of the fixity of species, but in several works from 1801 to 1807 he set forth his conclusions on the gradual transformation of species, and in 1809 he published his great work, *Philosophie Zoologique*, in which his conclusions on this and many other subjects of a philosophical nature are set forth in great detail and in a charming style. It is not possible in a brief sketch to do justice to this masterpiece. Fortunately it has been translated into English by HUGH ELLIOTT (MACMILLAN AND COMPANY, London, 1914) with a critical introduction of 92 pages, and is thus available to English readers. If this work had been available to DARWIN in a good translation, it is doubtful whether he would have written of it as he did in a letter of January 11, 1844, to J. D. HOOKER: "Heaven forfend me from LAMARCK'S nonsense of a 'tendency to progression,' 'adaptations from the slow willing of animals,' &c."

There is no question that with our present knowledge of the factors of evolution, much of LAMARCK'S theorizing is out of date, sometimes even absurd, but it is always presented with a wealth of observations and an open mindedness to objections which are worthy of DARWIN himself. His *Philosophie Zoologique* is a storehouse of information, but his speculations are lacking in a critical examination of causes, a method that could come only from more extensive knowledge of fundamental principles of heredity, development, and adaptation than were available in his time. Many of the "absurdities" attributed to LAMARCK by hostile critics are due to misunderstandings of his language. But there is no doubt that as the physicist, CARL BARUS, said of him, "LAMARCK'S genius was destitute of the instincts of an experimentalist."

By general consent of naturalists he is recognized as the most important advocate of evolution before DARWIN. PACKARD says, "He was undoubtedly the greatest zoologist of his time. . . . We owe to him the foundation and definition of invertebrates, infusorians, annelids, crustaceans, cirrhipedes, and arachnids." With his wide knowledge of plants as well as of animals he first recognized the fundamental likenesses of all living things, and in 1802 he proposed the name "Biology" for the science that deals with both plants and animals. In that year G. R. TREVIRANUS also introduced the same word.

LAMARCK was a thoroughgoing evolutionist and held that man and all his faculties were products of evolution. He said, "At bottom the *physical* and *moral* are without doubt one and the same thing." Instead of viewing more complex phenomena from the standpoint of man, they should be viewed from that of less perfect forms. An examination "should have been made of the progression from the simplest animal up to man. . . . With regard to living bodies it is no longer possible to doubt that nature has done everything little by little and successively." Finally, regarding the origin of nature he wrote, "Since I can have no positive knowledge on this subject I prefer to think that the whole of nature is only an effect: hence I imagine and like to believe in a first cause or, in short, a supreme power which brought nature into existence and made it such as it is."

LAMARCK'S relations to modern genetics are remote and incidental. Thirty years before the work of SCHLEIDEN and SCHWANN he devoted a whole chapter of his *Philosophie Zoologique* (Chap. 5, Part II) to "Cellular tissue, regarded as the matrix in which all organization has been cast." It contains a remarkably full anticipation of what was later called "the cell-theory." He adds that, "Since the year 1796 I have been accustomed to set forth these principles in the first lessons of my course." He regarded life as "organic movements resulting from the action of a stimulating cause which excites them. This cause resides in subtle expansive fluids which penetrate the bodies which they animate." In accordance with this conception he regarded the unfertilized egg as not living and that at fertilization a subtle vapor or fluid escapes from the fertilizing material, which under the influence of gentle warmth, makes it live. He firmly believed that habits may be inherited and become instincts and that the effects of use and disuse may be inherited. He lists many instances of such inheritance, no one of which would now be accepted as proof. In general he and his more recent followers, the Neo-Lamarckians, have shown a general tendency to confuse cause and effect.

However this controversy over the inheritance of acquired habits may be settled, it is well to consider seriously the question whether or not the mutations which are now regarded as the materials for evolution are not themselves, like all other physiological reactions, responses of living matter to stimuli, and if so, whether or not such responses involve differential sensitivity and selective reactivity, as is the case in many physiological reactions.

Perhaps the most direct association of LAMARCK'S name with modern

genetics is in connection with *Oenothera Lamarckiana*, but even here explanations and qualifications are necessary. According to BRADLEY M. DAVIS (1927), the plant which LAMARCK named *Aenothera* (*grandiflora*) in 1798 (?) and which was renamed *Oenothera Lamarckiana* by SERINGE in 1828 is a form of *Oenothera grandiflora* Solander (1789), while the plant made famous by DEVRIES is probably a hybrid and should be known as *Oenothera Lamarckiana* DeVries.

EDWIN G. CONKLIN

(The cost of the frontispiece and the accompanying biographical sketch has been met by contributions from the Editorial Board.)

GENETIC DATA ON THE TWO RACES OF COLIAS CHRYSOTHEME IN NORTH AMERICA AND ON A WHITE FORM OCCURRING IN EACH

WILLIAM HOVANITZ
California Institute of Technology, Pasadena, California
Received April 13, 1942

INVESTIGATIONS on the population structure of *Colias chrysotheme* in North America have been made with a view toward determining the genetic and ecologic relationships of the three color phases existing in the wild—namely, the orange, the yellow, and the white.

A deduction derived from distributional data alone has been formulated—namely, that the color phases of butterflies are purely expressions of a different genetic adaptation to the environment (HOVANITZ 1940, 1941). According to this hypothesis, the colors are by-products of the adaptation, perhaps being alternative metabolic materials derived from the varying growth processes, themselves under genetic control.

The present physiological and genetic data on the color phases of *Colias chrysotheme* suggest that the earlier view is essentially a correct one.

HISTORICAL ORIENTATION

At the time that this work on the three color phases was initiated in 1940, one geographical part of the yellow form was considered by many authors to be a species separate from the orange form. Another geographical part was considered to be merely a seasonal form or alternate form of the orange species. GEROULD (1923) had reported crosses made between the former two "species." He has since given more complete data on these results (1943).

EDWARDS (1868–1893) first bred yellow forms from orange females and orange forms from yellow females; the interpretation he obtained from this work has had a profound influence on the taxonomic standing of the forms in later years and accounts in part for the present confused nature of the nomenclature. GEROULD (1943) has questioned the results obtained by EDWARDS and claims that they are due to a mixing of the broods of different females. He further does not find these results compatible with his own. The data in the present paper show that EDWARD's data are capable of experimental reproduction and that there is little reason for questioning them.

The orange and yellow phases of *Colias chrysotheme* are designated "races" (HOVANITZ 1943a) rather than species or subspecies because of the nature of the case. This term "race" is used in a non-committal sense, not necessarily meaning a category inferior to a species nor one superior to a subspecies. The color characteristic of these butterifles which permits the segregation into yellow and orange phases is found to be inseparable from some physiological characteristics—for example, a food requirement and diapause difference. These characteristics are found to be of great importance in the wild with respect to controlling the geographical and ecological distributions of the races and hindering their interbreeding. Nevertheless, though these characteristics

cannot be separated, many genes are recombined in interracial crosses, both in the wild and in the laboratory, accounting for a blending of the other characters of each.

One of the genes which is found to be freely interchangeable between the races is that controlling white female color. Because the white females are indistinguishable as to race in the hybrid crosses, as well as in more than three-fourths of the wild overlapping geographical range (HOVANITZ 1943b, 1943d), the genetic data on all the three forms must be considered together. (GEROULD (1943) has made an attempt at separating the white females of the two races by the grade of orange color in the hind wing cell spot. As will be shown later, this character varies according to the quality of modifying factors of the white gene present. The range of variation is as great within a race as between races. However, the results are not without significance; they show that in the hybrid cross, a segregation of modifying factors has occurred, these factors being different in the two strains. The presence of different modifying factors in the two races has been deduced also from other data (HOVANITZ 1943c).)

GEROULD (1923) has shown that the inheritance of the white female form in both races of *chrysotheme* is controlled by a dominant sex-limited gene. In the present paper, these data are compared with those of the author based upon new material. Though the conclusion of GEROULD as above is confirmed, it is believed that his theory of lethal factors linked with the recessive and dominant alleles is unsatisfactory. The additional information reported on here leads to the conclusion that some strains or populations possess different modifiers of this gene which control the lethal, semi-lethal, or viable nature of the homozygous dominants.

THE INTERRACIAL CROSSES

The essential visible difference between the yellow and orange races lies in the orange suffusion which is in the one and not the other on the upper surface of the fore wing. Though the color of the hind wing (upper side) cell spot, the color of the under side fore wing, and the color of the upper side hind wing may vary in unison with the color of the fore wing upper side, in some races there may be a difference only in the latter. This is true particularly for the females. A number of non-visible physiological differences have been found to be present between these races, but owing to the difficulty of determination in individual cases, mention of these will be made later.

An arbitrary scale of from (1) to (10) was arranged, giving at (1) a light yellow color with no suffusion of orange and at (10) a full orange suffusion on the fore wing, upper side. The scale is useful mainly for comparing butterflies bred at uniform environmental conditions or at least those which appear to have had fairly optimum conditions. The reason for this is that the amount of orange pigment is directly changeable according to the environmental conditions (HOVANITZ 1943b).

GEROULD (1943) has used a tint photometer with which the degree of red in the wing pigment was obtained in a standard area near the base of the fore wing. His units range from 0.1 for a light yellow butterfly to above 5.6 for the

orange. Approximate congruence may be obtained with GEROULD'S values by means of the following table. This is based upon some individuals sent him for determination and later compared with the present standard series. For the purposes of this paper, when GEROULD'S data have been mentioned his values have been translated into these standards.

PRESENT GRADE	GEROULD'S GRADE
1	0.1–0.7
2	0.7–1.0
3	1.0–1.3
4	1.3–1.7
5	1.7–2.0
6	2.0–2.2
7	2.2–2.9
8	2.9–3.4
9	3.4–4.0
10	4.0–5.6+

Table 1 shows the results of crosses between the orange and yellow races.

The segregations show that the factors controlling the orange coloration are multiple factor ones. The F_1 is normally exactly in between the two parental types. The F_2 present a wide range of variation nearly covering the range from 1 to 10. The backcrosses cover the grades between the parental types and, perhaps due to environmental shifting, somewhat beyond them. The broods of GEROULD were subject to the outside environment at Hanover, N. H., and show seasonal changes in the phenotype. The summer broods show more orange than the autumn ones. The wide range in the F_1 variability (from 3–7 or from 5–8) is certainly due to the changeable environnental effect. The shift in F_1 mean from grade 4 in two cases to 7 in other cases is due to seasonal climatic effects. The F_2 segregations likewise show a seasonal shift in the mean from grades 3 or 4 to 5 or 6. The greater variability of the F_2 is expected.

The backcrosses are all late season broods and would show the effects of cold on the phenotype. Pertinent here is the expected reduction in orange. The crosses $F_1 \times$ orange parent and the reciprocal all show a shift toward yellow beyond the expected F_1 grade. The color plates of GEROULD (1943) show that this is phenotypic. The segregation of the crosses by HOVANITZ grown at near optimum does not show this shift in phenotype.

The lack of F_3 crosses is apparently not so much due to sterility in the butterflies as to loss of stocks during the winter at Hanover or to difficulties with food at Pasadena. A brood was obtained from two individuals which had arisen from a backcross. The backcross individuals are therefore fertile.

Tests of sexual selection made between the yellow and orange race individuals were found to have little value. This is due to the problems arising from the larval diet. Difficulty was encountered in getting orange $\times$ yellow matings and likewise even yellow $\times$ yellow. Orange $\times$ orange (including the white female) took place readily. This will be discussed under larval-diet sterility and sexual apathy. In the crosses made at Pasadena, no sterility has been found between yellow race and orange race individuals which cannot be traced to larval diet difficulties.

TABLE 1

Crosses involving the orange and yellow races. The source of the data is GEROULD *or* HOVANITZ *as shown by the letters.*

SOURCE OF PARENTS	SEX	GRADES 1	2	3	4	5	6	7	8	9	10	W	TOTAL
F1 A Tempe, Ariz. orange×Hanover, N.H. yellow	G male						2	24	3				72
	female						15	19	3			36	71
F1 B Hanover, N.H. yellow×Tempe, Ariz. orange	G male					8	12	6	2				122
	female					1	1	19	3			0	40
F1 C Hanover, N.H. yellow×Tempe, Ariz. orange	G male					4	9	30	2				45
	female							4	5			7	16
F1 D Tempe, Ariz. orange×Hanover, N.H. yellow	G male			3	7	8	0	4					20
	female			1	4	4	0	1				24	34
F1 E Round Valley yellow×Round Valley orange	H male				2	13							15
	female												0
F1 F Tempe, Ariz. orange×Hanover, N.H. yellow	G male												1
	female			6	3	2							11
F1 G Tempe, Ariz. orange×Hanover, N.H. yellow	G male				14	11							25
	female											3	8
F1 H Round Valley yellow×Round Valley orange	H male												
	female												
F2 A Grade 7 from F1 B×Grade 8 from F1 B	G male		2	0	0	3	0	2	1				6
	female		1	1	2	0	0	1				0	6
F2 B Grade 5 or 6 from F1 B×same	G male		1	3	1	2	5	1	0	4			12
	female	1	2	1	3	1	2	1				0	22
F2 C Grade 6 from F1×Grade 6 from F1 A	G male	3	3	4	2	1							13
	female				3							3	6
BC A Grade 7 from F1B×Hanover, N.H. yellow	G male	7	2	0	3	5	3						24
	female	2	5	3	16	7	3					0	36
BC B Intermediate×orange from Tempe, Ariz.	G male			2	23	7	7	8	2	1			62
	female											0	29
BC C Tempe, Ariz. w×Grade 7 from F1 A	G male			2	5	2	4	11	4	2			33
	female											39	39
BC D Tempe, Ariz. w×Grade 7 from F1 A	G male			3	7	3	4	17	5	1			36
	female			0	4	3	0	4	0	2		15	28
BC E Rio Hondo w×Grade 5 from F1 E	H male							1	0	1	6		8
	female							2	4	1		5	12
BC F Rio Hondo w×Grade 6 from F1 E	H male						1	0	7	3	19		30
	female							2	5		1	3	11

STERILITY AND SEXUAL APATHY INDUCED BY LARVAL DIET

The breeding of the yellow and orange races of *Colias chrysotheme* in North America has shown that the two races differ in their food requirements. Sterility, sexual apathy, high mortality, and phenotypic alterations are the result of interchanging the normal food of the two races.

Owing to the fact that females are often not too particular about the leguminous plants upon which they lay their eggs, a long list of larval food plants has accumulated in the literature. This list is not very different for the two races. Caterpillars presumed to be of either race (they are indistinguishable) have been taken on most plants on the list. In some cases larvae have been bred on the plant.

The recorded larval food plants of the orange race in the wild are as follows: alfalfa (*Medicago sativa*, introduced), Trifolium (including *repens*, *pratense*,

etc., which are introduced and native clovers), Lupines (*Lupinus* sp.), vetches (*Vicia* sp.), loco-weed (*Astragalus* sp.), sweet white clover (*Melilotus alba* and other species), *Lotus* (*Hosackia*) *americana* and other species, various grasses.[1]

The author has observed in the wild the laying of eggs by the orange race on alfalfa, on white clover (*T. repens*) and native clovers, and on *Astragalus* sp., but not on red clover (*T. pratense*). Eggs and larvae have been taken from these same plants. Adults have been bred on alfalfa and on red clover. The adults were not fertile in the latter case.

Observations of oviposition were made at Round Valley in a field of mixed red clover and alfalfa of about equal proportions and height (one and one-half feet high). More than ten laying orange females were observed attaching their eggs onto the dorsal surface of alfalfa leaves, wholly ignoring for this purpose the red clover in the field. Each of these females was under observation for five minutes or more, and some eggs were collected. At the same time five yellow females were observed laying on the red clover in the same field. It is not known if, when red clover alone is present in a field, the orange females will lay upon it. SCUDDER (1889) says that the orange females ignore red clover. Caged females of the orange type will lay upon red clover in the laboratory with no difficulty—the eggs being laid on the dorsal surface of the leaves. This observation is factual though contrary to statements of others that the hairiness of the under side of the leaves prevents the eggs from being attached.

CLARK (1932) records eggs being laid by this species (*eurytheme*) on red clover and on sweet clover (Melilotus). However, according to the viewpoint at that time, the large, angular yellow butterflies present in the vicinity of Washington, D.C., were classifiable as *eurytheme* (or the orange form). It is now believed that the yellow phase in that locality represents the yellow race altered by repeated intercrossing with the orange. Therefore, it may have been yellow females which had laid the above eggs.

Larvae and pupae of the orange form bred on red clover are modified in coloration as compared with the same bred on alfalfa. The former have a bluish color to the blood, absent in the latter. The same difference has been noted in the yellow race but is not so striking. The effect of this color change of the blood is to give the larvae and pupae a blue-green color, as compared with a yellow-green color. The dung of the larvae of the former is also bluish and darker. It quite resembles the dried leaves of the red clover itself and is apparently derived directly from the food plant.

The listed food plants of the yellow race are the same as the orange race but with the addition of the following: Amorpha (indigo; this is the normal food plant of *Colias caesonia-eurydice*), *Baptisia tinctoria*, *Pisum sativum* (cultivated pea), Cytisus (Scotch Broom), Caragana. The latter three are introduced from Eurasia.

The yellow form from New England, Colorado, and Kansas has been bred (fertile) on white clover (*T. repens*) by GEROULD. From California (Mono

[1] Data from COMSTOCK 1927; FIELD 1938; HOLLAND 1931; WRIGHT 1905; SCUDDER 1889; EDWARDS 1868–1893; CLARK 1932, MS; GEROULD 1943; KLOTS 1930; GARTH 1935; COMSTOCK 1940, etc.

Lake and Round Valley), yellow forms have been bred by the author. These are fertile on red clover (*T. pratense*), on alfalfa and red clover in part, and alfalfa and white clover in part. In one case a fertile mating was made between a yellow male and a yellow female (Round Valley stock) bred wholly on alfalfa from which young larvae were obtained. They all died on alfalfa in the first instar. There is a possibility that had these larvae been on clover, they might have survived. Several stocks derived from New Hampshire wild yellow females were bred with difficulty on alfalfa at Pasadena. Some of these died in various larval stages—others nearly always went through to the imago but with phenotypic alterations. Of four matings made between such individuals, one female laid fertile eggs. Larvae from this set died in the first instar on alfalfa. The "yellow" individuals bred on alfalfa are usually of a phenotypically reduced sort. They are much smaller and have a "spring type" of pigmentation (HOVANITZ 1943b).

Larvae of the yellow races bred on alfalfa through several instars generally have a "sick" appearance. They have little vitality, their skin is wrinkled, and their color is a lighter-than-normal green. In one case where several of these "sick" larvae in the last instar were obviously not far from the point of death, they were removed from the alfalfa and placed on white clover. Four out of seven apparently recovered enough to pupate. One female imago was obtained. She was mated to an orange male bred on alfalfa and laid fertile F_1 hybrid eggs. Seven yellow males and nine females of the same brood bred wholly on alfalfa would not mate with their kind or with fertile "orange" individuals (reciprocal crosses). The one yellow female referred to above could not be mated to any of her yellow brothers bred on alfalfa, though the attempt was made for several days before introducing an orange male into the same cage. This female was phenotypically also the most nearly full-grown of all the individuals of that brood.

The F_1 intermediates are apparently fertile on both alfalfa and white clover, at least some individuals. GEROULD (1923, 1943) bred his F_1 (New Hampshire yellow × Arizona orange) on white clover, and though some difficulty was met in getting copulations with some individuals, many were fertile. All GEROULD'S strains of the yellow race were bred on white clover; the orange were partly on alfalfa (personal communication).

The F_1 of Round Valley stock was bred on alfalfa (parents were from alfalfa and red clover). At least two of these F_1's were fertile. In another F_1 cross (from yellow ♀ on alfalfa and white clover × orange ♂ on alfalfa), the adults seem to be normal. The eggs were fertile, and larvae resulted. The first third of the larvae (about 15) were grown on alfalfa. The second third were removed from alfalfa immediately after hatching from the egg to white clover. They all died on the white clover in four to five days in their second larval instar. The third portion of eggs grew to the imago on alfalfa.

The yellow female parent of the last F_1 discussed was the only female of her brood (about ten) which would mate with either yellow males (bred on alfalfa) or with orange males (bred on alfalfa). The others had a complete diet of alfalfa. This one female is the individual discussed just above which was bred partly on white clover.

The sexual apathy and sterility of yellow-race individuals bred on alfalfa may be illustrated by the following:

(a) Caged 5 white and 5 orange Garvey orange-race ♀ ♀ with 7 yellow-race (Round Valley) ♂ ♂. All were bred on alfalfa. In four days with excellent conditions no matings occurred. Orange race copulations may be expected to occur within a few hours, normally. The females were sexually very active, but the males were not.

(b) Replaced the above females by sisters, but in two days there were still no copulations.

(c) Caged three yellow sisters bred on alfalfa with four yellow males also bred on alfalfa (brothers of above). No copulations and little sex play.

(d) Caged yellow sister of above (from alfalfa) with Garvey (orange race ♂, brother of above ♀ ♀). Some sex play, especially by the male. A female was once noticed in typical mating pose with abdomen curved toward male and with wings spread. Contact was made with the male but no copulation.

(e) Single pair mating of same individual male in (d) with an orange sister occurred in one hour after exposure.

(f) The ten females in (a) were exposed to four brothers (orange). Three copulations occurred in the same day, two white females and one orange female.

Similar mating difficulties were found with F_2 and backcross intermediates. The yellow half (grades 1 to 5) of the segregation when bred on alfalfa are sterile or incapable of mating. This suggests that the diet necessity is directly correlated with the yellow race and cannot be separated from it.

The desire or lack of desire to mate is not sterility. However, three crosses (copulations) made between yellow ♀ ♀ and yellow ♂ ♂ from the grades 1–4 of an apparently F_2 segregation (bred on alfalfa) produced a few eggs. The eggs laid did not start development. Crosses made between grades 6–10 and white ♀ ♀ of the same brood were fertile on alfalfa (nine crosses made), and progeny were fertile.

Sterility was noticed in two crosses made between New Hampshire yellows bred on alfalfa, though fertile eggs were laid in a third case. The larvae died on alfalfa immediately after hatching.

Crosses were made between yellows arisen from a single pair mating, half of which were bred on alfalfa and half on red clover. Copulations could not be induced in individuals from alfalfa, but several were made between those bred on red clover. Inter-strain (food strain) copulations were difficult, since only one from several individuals occurred. No eggs were obtained. Interracial crosses were attempted with orange-race individuals bred on alfalfa. Combinations were made as follows (all individuals from these two strains unless noted):

A. yellow ♀ ♀ (alfalfa) with orange ♂ ♂ (alfalfa)
B. yellow ♀ ♀ (red clover) with orange ♂ ♂ (alfalfa)
C. orange ♀ ♀ (alfalfa) with yellow ♂ ♂ (alfalfa)
D. yellow ♀ ♀ (alfalfa but different stock) with orange ♂ ♂ (alfalfa).

Two copulations were obtained after two to three days: yellow ♀ (red clover)×orange ♂ (alfalfa); yellow ♀ (alfalfa from D)×orange ♂ (alfalfa).

Eggs from the former were fertile, larvae were obtained, and F_1 bred on alfalfa. Few eggs from the second cross were obtained, and these failed to start development (that is, remained yellow or white and then dried up).

Possible sexual selection of F_1 male hybrids against yellow females bred on alfalfa is illustrated by the following:

Five F_1 grade 5 and 6 intermediates (bred on alfalfa) were placed with two yellow females bred on alfalfa and three white females (orange race) bred on alfalfa. Two of the males mated the first day with two of the white orange-race females. The remaining three males, two yellow females, and one white orange-race female were kept together for five days more but with no further copulations. After the first day, the mated males were re-introduced with the remaining females but with no further copulations. The two white females laid fertile eggs (backcross).

The existence of yellow "sports" or aberrations within orange-race populations (HOVANITZ 1943b) indicates that the orange pigment may be eliminated in some cases in a way separate from that differentiating the races. It has been shown that there is a series of genes or alleles controlling orange pigmentation in pure orange-race populations (HOVANITZ 1943b). These are arranged in such a way as to give a continuous series from very orange butterflies to those completely lacking orange pigmentation. The latter are exceedingly rare in wild populations. The progeny of a yellow female of this sort bred on alfalfa were found to be fully fertile. So the food plant preference which is correlated with the color difference in the yellow-race is not so correlated in the yellow "sports" of the orange race.

Summary

It seems clear that the yellow race is sterile and sexually inactive when the larvae are bred on alfalfa but not so when bred on white or red clover. The same is true for the yellow end of the F_2 and backcross segregations. Much of the sterility and sexual apathy apparently obtained in these crosses (such as by GEROULD) may be related entirely to dietary requirements.

The orange race in one trial was sterile on red clover. Since it is not attracted to it in the wild, perhaps red clover is not a sufficient food for this race.

The yellow race is fertile on and well attracted to both red and white clovers. The orange race has not been tried in the laboratory entirely on white clover, but since the orange half of F_2 and backcross segregations grown on white clover by GEROULD have been at least partly fertile, it may be assumed that this food plant is satisfactory. Orange females lay on white clover in the wild.

Yellow "sports" in orange populations have the orange-race food preference.

DIAPAUSE

The yellow race during the middle of the larval stage is able to go into a diapause. This normally does not take place unless instigated by some environmental condition. Low temperatures, dried or frost-bitten clover leaves, the wrong food plant (such as alfalfa), and perhaps other things will cause the larvae to stop feeding and become dormant. This is true for larvae of New Hampshire yellow as well as for those from California and Colorado.

The orange race larva dies rather than becomes dormant when conditions become untenable. Cool temperatures which will induce diapause in the yellow race (0°C to 10°C) will merely slow up the development of the orange race. This fact was noticed by EDWARDS (1868–1893), but the significance was not apparent then. GEROULD (1943) has mentioned this diapause in his yellow material also.

Parts of intermediate broods, in which grades 1–5 individuals are expected, will go into diapause when bred on alfalfa just as yellow-race stocks do. However, it is not known that the F_1 will do so. Orange strains derived from the grades 6–10 of these broods have shown no diapause tendencies when bred on alfalfa. Apparently the diapause is correlated directly with the yellow color and the food preference. These characters do not seem to be interchangeable between the races.

However, the existence of yellow "sports" or aberrations within pure orange-race populations indicates that the diapause may be separable from the color difference. The eggs and larvae from one such individual (Grade 2) were bred out-of-doors (6°C night to 20°C day) on alfalfa in the cold of autumn. Under the same conditions, the eggs and larvae of several yellow females from the yellow-race population at Round Valley were bred. The second instar larvae of the latter went into diapause on and under the leaves of the red clover, whereas the former continued to grow to pupation and were partially killed by frost. This illustrates that the orange pigment may be eliminated without the necessity of introducing the controller of the diapause. This does not seem to occur in the yellow-race but only in the orange.

INTERMEDIATES IN WILD POPULATIONS

Considerable intermating occurs between the orange and yellow races where populations of each exist in the same place. The more intermixed the food plants of each race are, the greater the hybridization between the two. In fields where alfalfa, red and white clover are commingled, the amount of intercrossing is very high. Since females will generally mate immediately upon eclosion and rarely after the first copulation, the males which are present in the immediate vicinity of the newly eclosed female will be more likely to mate with her than males in adjacent fields. This would be true despite the flying of the females into adjacent fields. The important part of the intermixing of the adults must be the transfer of sperm from one race to the other. No amount of commingling after the mating period could have any effect on the genetic structure of the races.

Intermediates between the yellow and orange races have been found everywhere when the two races occur more or less together. Usually the habitats occupied by the races are different. An alfalfa field is usually a pure alfalfa field and can support only the orange race. Red clover fields are often pure red clover fields and can support only the yellow race. Astragalus, Vicia, etc., prefer dryer habitats than most of the clovers, and microhabitat differences will then arise between the races. It is possible that prior to the growing of clover and alfalfa together as a hay crop, there may not have been a great deal of inter-

crossing between the races. In the 1880's, however, EDWARDS (1884) bred orange specimens from yellow wild Colorado females and yellow specimens from orange wild Colorado females. He makes no mention of intermediates, and it is possible that these were either present but noted only as "*eurytheme*" or had died as larvae by reason of their lower viability. Table 2 shows the results of the breeding from wild females by EDWARDS. The material was bred on "clover," probably white clover, so the diet would have been satisfactory for either race.

TABLE 2

The nineteenth century records of EDWARDS *on intercrossing between the orange and yellow races in Colorado.*

PARENTS	YELLOW	ORANGE	WHITE
6 yellow ♀ ♀	97	0	0
1 yellow ♀	0	12	3
2 yellow ♀ ♀	25	5	0
1 orange ♀	3	5	0

Records of copulating pairs of the orange and yellow races are very rare. Most collectors have seen orange×orange, white×orange, yellow×yellow, and white×yellow pairs, but few have seen orange×yellow or the reciprocal. EDWARDS (1869–1893) gives the only record known in the literature of the 19th century: yellow ♀ ×orange ♂ in copulation in Illinois. At that time this locality was as far east as the orange race was to be found in fair numbers. CLARK (unpublished) notes that yellow×orange and reciprocal crosses were common at the time of the first influx of orange race into the vicinity of Virginia but are no longer so.

Copulated pairs of intermediate and parental types have been taken at Mono Lake and Round Valley as follows: yellow ♀ ×orange ♂, orange ♀ × orange ♂, white ♀ ×orange ♂, grade 6 ♀ ×orange ♂, yellow ♀ ×yellow ♂, yellow ♀ ×grade 6 ♂.

The relative frequency of the types means little, since there is reason to believe that more yellow females in the mating period are present at these localities than orange females.

Persons well acquainted with populations of this butterfly in areas where both races occur have not failed to note the blending of the characters of each. ELROD (1906) finds that in Montana there is no complete separation of the yellow form from the orange, since they blend one into the other. KLOTS (1930) finds the same true for Wyoming. Each of these authors however, has, found that there are definite broods of one race occurring in certain locations or seasons. ELROD finds that the yellow race is more restricted in distribution to certain areas, while the orange is everywhere. J. C. HOPFINGER (personal communication) has noted the same for north-central Washington. Apparently, a similar situation is true for Kansas (FIELD 1938). The results of a complete analysis of populations at Mono Lake and Round Valley, California, show the same to be true (HOVANITZ 1943c).

Intermediates have been obtained in the wild from as far north as British Columbia and at least as far south as California, New Mexico, and Georgia. They probably occur in Mexico as well. They are very common in eastern California and Nevada (Mono Lake, Round Valley, Honey Lake Valley, Modoc Co., Carson Valley), Great Salt Lake Valley (Utah), Montana, Idaho, Colorado, New Mexico, Kansas, and the vicinity of Washington, D.C.

A very early record for New England of an "orange *philodice*" is presented by SCUDDER (1889). This specimen is probably an intermediate between the races and was taken in Bangor, Maine.

Breeding from wild intermediates

Attempts were made to breed the progenies of many wild intermediate females on alfalfa. Many of these did not get far beyond the early larval stages, possibly due to dietary difficulties or lethal recombinations of genes (see egg counts later). The main conclusions to be derived from these broods are (1) that the wild intermediates may be F_1, F_2, backcross, F_3, F_4, F_n individuals, (2) that the wild intermediates which have laid any eggs at all have laid mostly fertile ones, (3) that the phenotype of the intermediate is true to its genotype, and (4) that the progeny give a range of variation as expected had the female mated with a given hypothetical male.

TABLE 3

Segregation in progeny of wild intermediate females or females which gave intermediate progeny; from Mono Lake and Round Valley, Calif.

SOURCE OF PARENTS	SEX	GRADES 1	2	3	4	5	6	7	8	9	10	W	TOTAL
Grade 1 R.V.×Grade 6 R.V. (wild cop.)	H male	2	0	0	1	1							4
	female		2	0	0	2						0	4
Grade 4 R.V.×? R.V. (prob. yellow)	H male	4	1	1	2	1							9
	female	1	0	2	2	0	1					0	6
Grade 6 R.V.×? R.V. (prob. yellow)	H male	1	0	3	1	2	1						8
	female	4	2	1	3	1	2					0	13
Grade 6 R.V.×? R.V. (prob. yellow)	H male	1											1
	female											0	0
White (2-2) M.L.×? M.L. (prob. yellow)	H male	6	5	0	0	2	6	4	10	16	21		68
	female	3	4	2	0	1	5	8	11	6	3	13	56
White R.V.×? R.V. (prob. yellow)	H male	2	0	0	0	0	0	0	0	0	5		7
	female											0	0
Grade 4 R.V.×? R.V. (prob. yellow)	H male	6	2	1	2	1							12
	female	2	3	3	0	1						0	8
Grade 5–6 R.V.×Grade 1–2 M.L.	H male	2										0	2
	female											0	0

Table three gives the grades of progeny bred from wild intermediate females. The numbers are small due to the fact that intermediates between grades 2 and 6 were usually selected as parents. Since only alfalfa was used as food, the mortality is very high. Likewise, the yellow end of the segregation would be expected to have a higher mortality than the orange end and the progeny would be expected to be sterile due to diet. The unknown males were probably of the yellow race judging from the segregation in the progeny and the fact that most males at Round Valley are yellow. The white female from

Mono Lake was probably an F_1 intermediate; her mate must have been that, too, in order to have given a range of intermediates and parental types such as that shown. The skewness of her curve (high on the orange side and low in the center) might be accounted for by sperm from several males, but the 13:43 ratio of white:orange-yellow could not be. It is more likely that half of the expected white females were of the yellow type and had died before the imago stage. The skewness of the curve is also best explained by high morality on the yellow side; the latter individuals which were present were sterile.

GENETIC DATA ON THE WHITE FORM

In the wild, individuals have been found with all yellow and orange pigment replaced by white, or with various degrees of substitution. White individuals exist most commonly in the female sex, but occasionally males have also been taken. A clear-cut segregation into white verus yellow or orange usually makes a classification of these forms quite easy. However, there is considerable fluctuation within each type, and some individuals exist which bridge the gap. The latter individuals have been observed only in the wild.

That the common white female variety of each of the yellow and orange races was a genetic variant was first noted by W. H. EDWARDS (1868–1893), though he did not understand the significance of it. EDWARDS bred both yellow and white progeny from a yellow female. The numbers in the brood, however, were too small to give much genetic information (see table 2).

MR. THOMAS E. BEAN in 1891 (see GEROULD 1923) bred the progeny of yellow and white wild females of *Colias christina* from Alberta, Canada. This species shows a much higher degree of intermediacy between the colored forms than do any of the races of *Colias chrysotheme* other than *vitabunda*. For the most part, however, the progeny could be classified as white or yellow. Except for the progeny of one female of an intermediate type between orange, yellow, and white which gave 16 progeny of various grades of the three colors, the results seem comparable to the genetic results in the *chrysotheme* group to be discussed later.

FROHAWK (1901) bred progeny in the ratio of 110 white ♀ ♀ : 125 orange ♀ ♀ from four white wild females of *Colias crocea = edusa* (European species). This is probably a 1:1 ratio.

MAIN and HARRISON (1905) bred progeny from a wild white female of *crocea* in the ratio 52 white ♀ ♀ : 19 orange ♀ ♀ (3:1 ratio).

PIESZCZEK (1917) has bred butterflies from white females of *Colias myrmidone* of Europe where the frequency of whites varies from more than 50 percent to close to 0 percent.

2 white ♀ ♀ (Upper Styria): 30 white:0 orange = 1:0 ratio
1 white ♀ " " : 18 white:17 orange = 1:1 ratio

(Majority of females in Upper Styria are white though yellows, oranges, and whites are common in the populations.)

5 white ♀ ♀ Graz = 23 white:19 red (orange) = 1:1 ratio

(White females at Graz are fairly rare. Population count of 33 red; seven intermediate; 11 white.)

GEROULD (1911, 1923) has studied the inheritance of a genetic factor controlling white wing color in females of *Colias philodice* (yellow from New Hampshire) and *Colias eurytheme* (orange from Arizona). In the earlier paper, the author showed that the gene was dominant, and he postulated homozygous lethality due to the difficulty of getting 3:1 ratios (nearer 2:1 was the result). Later, this was assumed to be due to the existence of an occasionally linked lethal recessive factor, for broods were obtained having 3:1 ratios as well as 100 per cent white progeny.

Likewise, unexpected ratios, such as 100 percent white females from a supposedly heterozygote×heterozygote led GEROULD to postulate a lethal factor associated with the recessive factor for yellow. The data of GOLDSCHMIDT and FISCHER (1922) on a similar sex-limited gene in *Argynnis paphia* of Europe gave similar results; it was concluded that the dominant gene was usually linked with a recessive lethal. The present author believes that the evidence needs to be reconsidered and that the reason for these aberrant results probably lies in the effect of modifying factors.

From ratios obtained from wild females alone, it is clear that the gene controlling white female color is dominant, in both the orange and yellow races. This conclusion has been arrived at by GEROULD (1923). White females have never failed to produce at least some white female progeny when the total number of female progeny was large. Only one wild female of this species has yet produced a 100 percent white female brood which is statistically significant and this one from a locality where the white frequency was 71 to 74 percent of the females in the population. The two white females of the *Colias myrmidone* of Europe produced all white broods; these also came from a population having a high white frequency.

It may be assumed fairly safely that most wild females have mated but once. However, old females have been found in copulation. Old females have been mated more than once in the laboratory, but only when the supply of sperm has been exhausted; the females also have exhausted their supply of eggs.

Males are apparently not affected phenotypically by the genetic controller of the white color in these females. However, the existence of very rare white males in wild populations suggests that there are genes which do control this character or a similar one on a bisexual basis. Except for one case which will be brought up later, no white males have appeared in any of the cultures. This one exceptional case is obviously due to a different recessive gene with a much more drastic effect than the dominant one.

Though the males are indistinguishable phenotypically, they apparently carry the gene for female whiteness in the same manner as do the females. The following genotypes should, therefore, appear in wild populations in various frequencies providing that none are lethal, notwithstanding the fact that visibly the males are indistinguishable: yellow or orange ♀ ♀ *ww*, white ♀ ♀ *Ww*, *WW*, yellow or orange ♂ ♂ *ww*, *Ww*, *WW*.

Individuals giving genetic results expected of all the above genotypes have been tested in either the yellow or orange races with the one possible exception of the homozygous dominant male. The difficulties in making the desirable

tests of individuals have their foundation in the laborious work involved in breeding the material. Without tremendous facilities, it is impossible to carry the larvae of many crosses through at any one time. Ten full grown larvae will consume a two foot high alfalfa plant in one to two days at 25°C. Also, since the genotype of a male cannot be told phenotypically, one must partly rely upon a lucky choice for an individual.

Without a narrative of minute detail on all the crosses made in testing the genotypes of the above forms, it will be impossible to describe the pedigrees of all the crosses made. Of course, this will eliminate desirable detail, especially where the small ratios are concerned. The latter have been summarized in the places where they seemed to fit best according to their pedigree.

Knowledge of the existence of all the various combinations of genes is desirable from the point of view of the genetics of wild populations. For that reason, the ratios from wild females are compared with those from laboratory ratios. The wild females from which these ratios have been derived have come from many geographical sources—namely, Hanover, N.H. (yellow-race), Pueblo, Colorado (yellow- and orange-races), Centennial, Wyoming (yellow-race), Tolland, Colorado (yellow- and orange-races), Manhattan, Kansas (yellow- and orange-races), Kearney, Nebraska (yellow-race), Round Valley, California (yellow- and orange-races), Mono Lake, California (yellow- and orange-races), Tempe, Arizona (orange-race), Rio Hondo (near Los Angeles), California (orange-race), Garvey, California (orange-race), Westley, California (orange-race), Elsah, Illinois (orange-race), San Fernando Valley, California (orange-race), Bouquet Canon, California (orange-race). Some data are from GEROULD (1923) and EDWARDS (1869–1893); the remainder from cultures of the author. The data indicate that the genetics of the variations are comparable in all localities. For specific details on geographical distributions of the races and frequencies of the white forms, maps are given in another article (HOVANITZ 1943d).

Granting the dominance of the white allele, the ratios from yellow or orange wild females giving all orange or yellow female progeny are evidence of crosses in the wild involving a homozygous recessive female and a homozygous recessive male. These total 0 white:505 yellow in 31 broods, 0 white:248 orange in 18 broods, or an interracial total of 0 white:753 yellow-orange in 49 broods. Laboratory crosses giving the same ratio total 0 white:216 yellow in ten broods, 0 white:696 orange in 18 broods, or a grand total of 0 white:912 yellow-orange in 28 broods.

Ratios of 1:1 from orange or yellow females would suggest the cross recessive female×heterozygous male. Of wild ratios of this type there are but two in the yellow-race totaling 8 white:11 yellow. In the orange-race there are several, 28 *w*:18 *or*, 13 *w*:17 *or*, 12 *w*:10 *or*, 21 *w*:18 *or*, 21 *w*:29 *or*, 20 *w*:23 *or*, 13 *w*:15 *or* in a single brood ratios and 9 *w*:13 *or* in 4 small broods, a total of 137 white:143 orange females.

The lack of F_1 ratios from wild yellow or orange females giving much more than a 1:1 ratio may be taken as evidence against the existence of a yellow

or orange heterozygote; were the latter in existence, 2:1 or 3:1 ratios would occur in the F_1 from wild material more frequently.

There are many small broods close to 1:1 or 0:1 but in which the male is known to have been a heterozygote. These have been included in the following totals for 1:1, 13 *w*:14 *y*, 7 *w*:5 *y*, 19 *w*:19 *y*, 28 *w*:28 *y*, 59 *w*:76 *y*, 1 *w*:2 *y*, 10 *w*:12 *or*, 7 *w*:14 *or*, 30 *w*:14 *or*, 43 *w*:35 *or*, 24 *w*:20 *or* (in six broods), 5 *w*:14 *or*, giving a total for the two races of 216 white:246 yellow-orange.

These data show that all ratios obtained from yellow or orange females are either 0:1 or 1:1 The 30:14 ratio in one case may be significantly different or not. The history of this ratio is given in table 4 (from GEROULD 1923, reorganized).

TABLE 4

Pedigree involved in the 30 : 14 ratio of GEROULD.

	WHITE ♀♀	YELLOW ♀♀	♂♂	EXPECTED
A Wild white ♀	15	13	30	1:1
B1 *w*♀ × *y*♂ from A	38	22	63	2:1 or 3:1
B2 *w*♀ × same *y*♂ as in B1	13	8	23	2:1 or 3:1
B3 *y*♀ × same *y*♂ as in B1	30	14	27	1:1
B4 *y*♀ × *y*♂ from A	19	19	46	1:1
B5 *w*♀ × *y*♂ same as in B4	3	3	4	2:1 or 3:1

In all cultures which are normal, males have a greater chance of survival over the females due to their faster development. When culture conditions are poor, it is the females which suffer most. This always gives a higher frequency of males in the cultures. In the brood B3 (Table 4), the males are about half that which would normally be expected, whereas the other broods are normal. It would appear that either an error has been made in recording the data or that something has happened to sex determination so that about half the normally expected males have become white females. Or possibly the unusual rare ratio has appeared as expected on statistical grounds.

Part of the data given above for the homozygous recessive female is equally applicable as evidence for a homozygous recessive male. All the 100 percent yellow or orange female broods must have had a homozygous recessive male parent.

Ratios obtained from white females which approximate 1:1 suggest that the male was homozygous recessive (and the female heterozygous). One to one ratios from wild white females of the yellow race are as follows: 22 *w*:28 *y* 15 *w*:13 *y*, 13 *w*:11 *y*, 8 *w*:17 *y*, 28 *w*:21 *y*, 7 *w*:16 *y*, 30 *w*:38 *y* (in seven broods), or a total of 124 white:144 yellow. One to one ratios from wild white females of the orange race are as follows: 29 *w*:24 *or*, 27 *w*:20 *or*, 13 *w*:15 *or*, 62 *w*:53 *or*, 16 *w*:8 *or*, 6 *w*:13 *or*, 20 *w*:15 *or*, 14 *w*:10 *or*, 16 *w*:6 *or*, 18 *w*:21 *or*, 32 *w*:20 *or*, 4 *w*:0 *or* (in two broods), or a total of 257 *w*:205 *or*. This gives a grand total for the two races of 381 white:349 yellow-orange.

Ratios from laboratory crosses involving a white female and where a 1:1 is expected are as follows: 28 *w*:38 *y*, 14 *w*:10 *y*, 65 *w*:69 *y*, 20 *w*:13 *y*, 6 *w*:11 *y*, 21 *w*:17 *y*, 13 *w*:12 *y*, 14 *w*:9 *y*, 8 *w*:8 *y*, 30 *w*:35 y (in ten broods), or a total of 219 *w*:222 *y* in 19 broods. These same ratios from orange-race white females are as follows: 16 *w*:11 *or*, 28 *w*:29 *or*, 9 *w*:10 *or*, 11 *w*:11 *or*, 6 *w*:13 *or*, 1 *w*:13 *or*, 27 *w*:22 *or*, 15 *w*:13 *or*, 43 *w*:35 *or*, 10 *w*:12 *or*, 20 *w*:16 *or*, 21 *w*:19 *or* (in seven broods), or a total of 207 white:204 orange in 18 broods. The interracial total is 426 white:411 yellow-orange

The evidence for the existence of the heterozygous white female has been partly considered above under homozygous recessive male×white female giving 1:1 ratio. No wild females representing crosses involving heterozygous white females and heterozygous males giving ratios approximating 2:1 or 3:1 have been taken in the wild, though such wild crosses must occur at a frequency varying directly with the gene frequency in the population.

Laboratory crosses giving such ratios or where those ratios are expected are as follows: In the yellow group—67 *w*:36 *y*, 49 *w*:25 *y*, 4 *w*:0 *y*, 17 *w*:0 *y*, 28 *w*:0 *y*, 38 *w*:22 *y*, 13 *w*:8 *y*, 95 *w*:28 *y*, 10 *w*:2 *y* (total=321 *w*:121 *y*—ratio=2.65:1). In the orange group—14 *w*:4 *or*, 9 *w*:0 *or*, 39 *w*:21 *or*, 83 *w*:31 *or*, 34 *w*:15 *or*, 37 *w*:22 *or* (total=216 *w*:83 *or*—ratio=2.60:1).

The heterozygote×heterozygote cross is expected to give a 3:1 ratio where the homozygous dominant is viable but indistinguishable from the heterozygote. Instead, however, the ratios vary from 3:1 to 2:1 with an average of 2.65:1 in yellow and 2.60:1 in orange. These averages, of course, mean only that neither 2:1 or 3:1 ratios are exclusively obtained. The production of 2:1 or 3:1 ratios seems to run in certain families. This was true also in GOLDSCHMIDT and FISCHER'S work on *Argynnis paphia* and its melanic mutant, where an average of 2.57:1 was obtained. Some families of the latter species gave 3:1, others 2:1, leading GOLDSCHMIDT to predict that a lethal recessive factor was associated with the dominant gene in certain cases and absent in others. In the more highly inbred strains, the 3:1 ratio was obtained, whereas 2:1 was obtained in relatively slightly inbred or F_1 from wild strains. As mentioned above, GEROULD (1911) first assumed that the homozygous dominant was lethal on the basis of 2:1 ratios in three broods. Later (1923), he postulated a recessive lethal factor which was eliminated in certain strains, because 3:1 and wholly white strains were obtained between 1911 and 1923. He also postulated a recessive lethal factor linked with the recessive yellow gene due to some anomalous broods of 2:1 where 1:1 was expected. The history of one of these broods is illustrated in Table 5.

It is seen that the total of the ratios for all three white sisters mated to the same male is 103:82, though one of these white females gave 67:36. Yellow sisters of these white females gave with the same male a ratio of 0 *w*:12 *y* ♀ ♀. The probability that the latter ratio is 1:1 is .0002, or quite unlikely. Therefore we may assume that the male is homozygous recessive. It should give a 1:1 ratio with heterozygous females. The ratio 103:82 has a standard error of 6.8. Since the deviation from the expected ratio is 10.5 (considerably less than twice the standard error), the difference need not be taken seriously.

In the specific case of the ratio 67:36, the standard error is 5.8 and the deviation from the 1:1 ratio is 15.5. The actual deviation is, therefore, just over twice the standard error. It may be significant. However, the assumption of a linked lethal factor with recessive yellow is not necessary here.

TABLE 5

Pedigree involved in a 2:1 ratio of GEROULD.

	WHITE ♀♀	YELLOW ♀♀	♂♂	EXPECTED
A wild white ♀	3	4	1	1:1, 3:1
B1 *w* ♀ from A×*y*♂ from A	28	38	64	1:1, 3:1
B2 *w* ♀ from A×*y*♂ same as in B1	8	8	28	1:1
B3 *w* ♀ from A×*y*♂ same as in B1	67	36	77	1:1, 2:1
total from 3 white ♀♀	103	82	167	1:1
B4 *y* ♀ from A×*y*♂ same as in B1	0	7	13	0:1
B5 *y* ♀ from A×*y*♂ same as in B1	0	5	3	0:1
total from yellow ♀♀	0	12	16	0:1

All F_1's from orange or yellow females that give white progeny in a 1:1 ratio are evidence that yellow or orange males are heterozygous for the dominant gene. These are listed in the section on homozygous recessive females. Other ratios of 3:1, 2:1 and some 1:0 could also be due to a heterozygous male but are listed elsewhere.

A homozygous dominant white female should give 100 percent white female progeny with any male. There are several broods of small number which have all white females; actually a ratio of 18 *w*:0 *or* has a probability of .0056 of being a 3 *w*:1 *or* ratio. Despite this, some low ratios are listed here. Only one ratio of many all white females from a wild female is known. Of course the female might have been an heterozygote which had mated with a homozygous dominant male. The same is likewise true of the laboratory crosses; whether the male or female was homozygous is unknown. No all-white brood has yet been obtained from an orange or yellow female (except possibly one); such a ratio would suggest a homozygous dominant male was involved. The following progenies had only white offspring: 19 *w*:0 *y*, 28 *w*:0 *y*, 24 *w*:0 *y*, 15 *w*:0 *y*, 10 *w*:0 *y*, 10 *w*:0 *y*, 21 *w*:0 *y*, 2 *w*:0 *y*, 2 *w*:0 *y*, 4 *w*:0 *y* (Total=135 *w*:0 *y*); 9 *w*:0 *or*, 25 *w*:0 *or*, 15 *w*:0 *or*, 12 *w*:0 *or* (Total=71 *w*:0 *or*). This gives a grand total of 206 white:0 yellow-orange. Broods of all-white females from wild females are: (1) from orange female—11 *w*:0 *or*, (2) from white females—7 *w*:0 *or*, 11 *w*:0 *or*, 28 *w*:0 *or* or a total of 46 white:0 orange.

The accompanying pedigree (Table 6, reorganized from GEROULD, 1923) is given to indicate the possible effect of selection of modifying factors upon the lethality of the homozygous dominant. When the material is inbred, white females are apparently more abundant in the crosses than when outbred to wild material (where the white frequency is low).

TABLE 6

Pedigree showing the effect of source of the genetic strain upon ratios of white to yellow females (reorganized from GEROULD).

A WILD WHITE ♀ (HANOVER, N.H.)	WHITE ♀♀ 1	YELLOW ♀♀ 3	♂♂ 5	EXPECTED
Crosses from A				
B1 *w*♀ × *y*♂	95	28	130	3:1
B2 *y*♀ × *y*♂ (same as B1)	28	28	77	1:1
B3 *y*♀ × *y*♂	59	76	146	1:1
B4 *y*♀ × *y*♂	0	118	127	0:1
Crosses from B3				
C1 *w*♀ × *y*♂	14	10	20	1:1
Crosses from B1 (unless noted)				
D1 *w*♀ × *y*♂	24	0	17	1:0
D2 *w*♀ × *y*♂	2	0	2	
D3 *y*♀ × *y*♂ from B4	0	13	21	0:1
D4 *w*♀ × *y*♂	10	0	7	1:0
Crosses from D4				
E1 *w*♀ × *y*♂	21	0	29	1:0
E2 *w*♀ × *y*♂ (same as E1)	4	0	5	1:0, 3:1
E3 *w*♀ × *y*♂	2	0	8	1:0, 3:1
Crosses from D1				
F1 *w*♀ × *y*♂	1	0	1	
F2 *w*♀ × *y*♂ same as in F1	15	0	2	1:0
F3 *w*♀ × *y*♂	10	0	14	1:0, 3:1
F4 *w*♀ × *y*♂ same as in F3	2	0	4	
Crosses from F2 (unless noted)				
G1 *w*♀ × wild ♂	6	11	22	1:1
G2 *w*♀ × wild ♂	65	69	217	1:1
G3 *w*♀ × wild ♂	1	1	1	
G4 *w*♀ × wild ♂	3	4	9	
Crosses from G2				
H1 *w*♀ × *y*♂	10	2	19	
H2 *w*♀ × *y*♂	5	6	16	
H3 *w*♀ × *y*♂	49	25	80	2:1
H4 *w*♀ × *y*♂	20	13	40	
H5 *w*♀ × *y*♂	3	3	8	
H6 *w*♀ × *y*♂	14	9	45	
H7 *w*♀ × *y*♂	13	12	26	
H8 *w*♀ × *y*♂	21	17	36	1:1
H9 *w*♀ × *y*♂	28	0	30	1:0
total	163	87	300	2:1

TABLE 6 (*continued*)

Pedigree showing the effect of source of the genetic strain upon ratios of white to yellow females (reorganized from GEROULD).

A WILD WHITE ♀ (HANOVER, N.H.)	WHITE ♀♀ 1	YELLOW ♀♀ 3	♂♂ 5	EXPECTED
Crosses from H_3				
I_1 w♀ × y♂	0	2	1	
I_2 w♀ × y♂	19	0	10	1:0
I_3 y♀ × wild y♂	0	20	2	0:1
Cross from H_5 and H_7				
J_1 y♀ from H_5 × y♂ from H_7	0	8	7	0:1

B_1 is a definite 3:1 ratio rather than 2:1 as often occurs in material from a locality where the white frequency is low. B_2 shows that the male used in B_1 was heterozygous. The higher number of females of yellow color in B_3 is hardly significant. B_4 as well as B_2 and B_3 show that the yellow females are certainly homozygous recessive. The male in C_1 is surely a heterozygote. D_1 to D_4 show that the males of B_1 as well as the white females are homozygous or heterozygous dominant. The yellow female in D_3 from B_1 is definitely homozygous recessive. E_1 to E_3 show the existence of homozygous and heterozygous dominants in D_4. F_1 to F_4 shows the same less definitely for D_1. In G_1 to G_4 the introduction of wild males which have an 83 percent chance of being homozygous recessive has eliminated homozygous dominants. The intercrossing of heterozygotes in H_1 to H_9 has given at least one 2:1 ratio. The 28:0 ratio in H_9 is an enigma. The I_2 ratio suggests that there were homozygous dominants in the 49:25 ratio of H_3.

So far we have been considering the white females as phenotypically identical, but this is not true. Some are whiter than others, have the melanic pigmentation reduced, have the orange cell spot of the hind wing reduced to a pale yellowish color, and have the yellow-orange pigment of the under side hind wing and apex fore wing exceedingly light in color. These differences are genetic. In view of the fact that there is no sharp delimitation between the lightly pigmented individuals and the darkly pigmented ones, it was not possible to segregate them into heterozygous and homozygous dominant on these characters alone.

A series of six standards was made of each of two of these characters—namely, darkness of orange pigment in (1) the hind wing cell spot and (2) the under side pigmentation. A formula is used to designate any one individual with respect to grade of these characters, for example: (1–1) = grade 1 in both characters; (2–5) = grade 2 in cell spot pigmentation, grade 5 in under side pigmentation, etc.

The crosses shown in Table 7 were made in order to test the genotype of different white females of these types. A single male was used with several females, since the phenotypes of the males are the same. The results show that decidedly variant ratios are obtained from the different white females mated to a single male. Cross H shows that the male chosen was a heterozygote. Crosses C and G suggest that the white females (3–3) and (2–1) were homozygotes.

Cross E suggests that the female (5–5) was a heterozygote but that the homozygote white females did not appear in the progeny. However, the probability for a 2:1 ratio is .1131 and for a 3:1 ratio it is .0829—that is, either is not unlikely. Cross F (5–5) and A (2–2) are the same as E.

TABLE 7

Pedigree involving white females having different modifiers for white color. All the females listed have been mated to the same male (bred from wild pupa from the San Fernando Valley, September 23, 1942).

♀ parent	WHITE ♀♀	ORANGE ♀♀
A white ♀ (2–2) bred from egg from Westley, Calif.	37	22
B white ♀ (1–1) same source as male, all died	—	—
C white ♀ (3–3) same source as male	15	0
D white ♀ (5–3) intermediate white-orange, all died	—	—
E white ♀ (5–5) same source as male	34	15
F white ♀ (5–5) same source as male	14	4
G white ♀ (2–1) same source as male	25	0
H orange ♀ (6–6) same source as male	10	12
I orange ♀ (2–1) same source as male, all died	—	—

More detailed and complete data are needed on the relation between the degree of coloration of these characters and the genotype of the individual. It may be that the whiter individuals are the homozygotes, though the results above seem to indicate that this is not always true. A review of the percentage of whites and its relation to the minor characters in wild populations will be given elsewhere. There is no sharp distinction between these minor variations but rather a continuous range of variability. However, populations in which the white female is abundant have a higher percentage of those individuals having formulae running from 1–1 to 3–3 than populations where the frequency of whites is low. It may be that homozygotes are more viable under the supplementary action of other genes which, on the side, affect the color of the cell spot and under surface.

Table 8 gives a pedigree illustrating the shift of ratios from white to orange and the reciprocal, depending upon the source of the material.

Parent A or its mate may have been homozygous white or heterozygous white. Round Valley had more than 50 percent females white in the orange race at that time. The male and females chosen for A1 might also have been heterozygous or homozygous for dominant white. The progeny of A1, therefore, could have been homozygous white, heterozygous white or some males might have been homozygous recessive.

Parent B was a wild intermediate, having the anterior half of the fore wings white, the rest of the wings normal yellow-orange. It probably was genetically homozygous recessive and had mated with a similar male.

Parent C was a very pale yellow-orange female. It was for this reason that it was bred. Its F_1 and F_2 progeny were full orange, however. An 11:0 ratio obtained from it suggests that it might have mated with a homozygous dominant

male. The probability that 11:0 could have been the ratio of a *ww* ♀ × *Ww* ♂ is .0005, not very likely but still possible. Its source (San Fernando Valley) is not the place where homozygous dominant males are likely to be very common.

Culture D5 gives a ratio well within the expected for a 3:1 ratio (probability .0535); both male and female were probably heterozygotes.

TABLE 8

*Pedigree showing the shift of ratios from white to orange depending upon the strain used for the female.**

	WHITE ♀♀	ORANGE ♀♀	♂♂	EXPECTED
A Wild white ♀ from Round Valley (3)	7	0	18	
A1 white ♀ × *or* ♂ from A	12	0	+	
B Wild intermediate white-orange ♀ from San Fernando Valley	0	4	7	
C Wild orange ♀ from San Fernando Valley	11	0	16	
All from C unless otherwise noted				
D1 white ♀ × *or* ♂				
D2 white ♀ × *or* ♂				
D3 white ♀ × *or* ♂				
D4 white ♀ × *or* ♂				
D5 white ♀ × *or* ♂ (same as in D1)	15	1	33	3:1
D6 orange ♀ from B × *or* ♂ (same as in D1)	5	21	57	1:1
D7 white ♀ from A1 × *or* ♂ (same as in D1)	29	0	35	1:0
D8 white ♀ × *or* ♂ (same as in D1)				
D9 white ♀ × *or* ♂ (same as in D1)				

* The point of including the lost or discarded material in this table is to accurately place it in pedigree form. The material was used for egg counts and will be considered in the text.

Culture D6 gives a ratio which should be 1:1 but instead is 5:21 (probability of being 1:1 is .0010). The chances are not great that it is 1:1, but since the male was the same as in D5 and is there obviously a heterozygote, we may assume that perhaps the high death rate of the cultures has created a selection for the orange females. It may be that in a given genic environment where modifiers have been selected for action with a particular gene, this gene may give one allele an advantage over the other. The environment, too, may select modifiers and thus allow a given allele to exist in a higher frequency in some places than in others. Correlated with this theory may be the probable death of homozygote dominants in some strains and their existence in others. Obviously, the problem needs study with a greater control of the genic environment as well as larger numbers of progeny brought to maturity and tested. It should be noted that the orange female parent of this culture is from a locality where white females are but 30 percent of the female population.

Culture D7 gives a ratio which is one hundred percent white. The male is the same heterozygote as in D5 and D6 but the female is from a strain produc-

ing in one generation seven white females, and 12 white females in the following. The female strain arose from a population where the white female frequency was more than 70 percent. The chances are great that the strain A1 had heterozygotes and homozygous dominants. The female of D7 must have been a homozygote unless the orange females expected were eliminated in the strong selection. Granting no selection, the 29:0 ratio has a probability less than .0001 of being a 2:1 ratio and of .0002 of being a 3:1 ratio.

It was in the progeny of the crosses (D) that the realization became firmly established that variations of the cell spot color and under side hind wing color of white females were genetic. Though most of the material was discarded before a check against the standard series was made, the data given in Table 9 were obtained.

TABLE 9

The grades of colors due to modifying factors or different genotypes on white females from different colored parents. Pedigree of the crosses is given in table 8.

CROSS	PARENTAL COLOR	RATIO	AVERAGE OF GRADES	NUMBER GRADED	HIGHEST GRADE	LOWEST GRADE
D5	white	15:1	1.65–1.75	8	3–3	1–1
D6	orange	5:21	5.00–5.00	2	5–5	5–5
D7	white	29:0	1.80–1.55	9	3–3	1–1

Apparently the white females from the orange parent, though by the same male, have decidedly a stronger orange cell spot and under side coloration. The white females from the white inbred parents have a very light spot and under side coloration. These data are also suggestive of a selection of modifiers for the better adaptability of the dominant gene in the genom, as has just been discussed.

FORD (1940) in *Abraxas grossulariata* has found that the yellow and white varieties are under unifactorial control and that the heterozygotes are intermediate. However, he finds that by plus and minus selection, these intermediates can be shifted from a continuous distribution with one parental type to the other. The shift, he indicates, is by altering the genome rather than the gene itself. Similarly, it may be that the heterozygote and homozygote dominant white females in Colias are distinguishable by the formula (1-1 being full homozygotes and 6-6 or 5-5 being heterozygotes) but that the variability in the genome accounts for the continuity between them, the gap between the homozygous recessive (orange phenotype) and the heterozygote being too great to bridge except in a few populations.

Conclusions

The preceding data illustrate that in both the yellow and orange races the heredity of the white female form is similar. (The data do not prove that the genes in the two races are homologous, but the similarity in physiological action and phenotypic effect combined with the genetic similarity makes it highly probable that they are.) In both races, the expected 3:1 ratio may be

reduced to 2:1 or some intermediate condition. Also there is a possibility that under a given gene environment one or the other of the homozygotes is at a disadvantage, thereby being selected out in a rigorous external environment. Apparently, the white gene is at its best in a gene environment such as that existing where the white gene is most abundant. The evidence suggests that any experiment designed to test the differential ability of the white versus orange or yellow females to survive must be combined with an intensive study of modifier selection.

From the standpoint of genetics of wild populations, when comparing the gene frequency in different geographical regions, the different ability of one genotype to survive (as for example, homozygous dominant) may be different in different places. Since this is probably true in more cases than not, the study of wild gene frequencies must necessarily be much more complicated than in present generalized work on the subject.

EFFECT OF INTERRACIAL CROSSES ON THE INHERITANCE OF THE DOMINANT GENE FOR WHITE

The question of homologies of genes between the orange and yellow races has not been attacked as in Drosophila (for review see STURTEVANT and NOVITZKI 1941). However, the apparently normal segregation of the alleles in the interracial crosses suggests that the dominant white gene for female color is enough alike to be interchangeable in the races. The accompanying data in table 10 on segregations are partially taken from the general tables of GEROULD (1923).

Except for the one ratio of 13 white to 53 yellow and orange, ratios are normal. The abnormal ratio may be accounted for by differential mortality due to larval diet as discussed earlier.

The important point here is the fact that the white gene is transferred easily from one race to the other. Apparently, this is not true for all genes which differentiate the races. Therefore, by sufficient crossing between the races in wild populations the white gene frequency in the two races will reach the same equilibrium in a given area.

THE WHITE MALE

White males have been obtained in widely separated geographical regions: Batavia, New York (1891) (probably yellow race); Columbia, Missouri (orange or yellow race); Alstead, New Hampshire (probably yellow race); Finleyville, Pennsylvania (probably yellow race); Sarver, Pennsylvania (probably yellow race); "Western Pennsylvania" (probably yellow race); "Michigan" (orange or yellow race); Bouquet Canyon, California (orange race); Mono, California (orange or yellow race); two F_2 from orange female, Rio Hondo, California (orange race).

These are not all phenotypically identical. In addition to the white color on the wings in place of the normal yellow and orange, these males usually have white legs, palpi, antennae, and body. The male from Alstead, N.H., was not of this type. The Pennsylvania specimens were not examined nor is a

TABLE 10

Ratios involving dominant white for female color in interracial crosses, illustrating that the gene is segregated independently of the racial complex.

CROSS	♀ PARENT COLOR	WHITE ♀	YELLOW, INTER., ORANGE ♀♀	♀♀
Orange race×Yellow race	white	36	35	72
Orange race×Yellow race	white	3	5	25
Orange race×Yellow race	white	4	3	11
Orange race×Yellow race	3 *w*♀♀ total	43	43	108
Orange race×Yellow race	orange, yellow	0	11	1
Orange race×Yellow race	orange, yellow	3	1	1
Orange race×Yellow race	white	24	10	20
Orange race×F_1 intermediate	white	4	3	5
Orange race×F_1 intermediate	white	35	38	108
Orange race×F_1 intermediate	white	15	13	36
Orange race×F_1 intermediate	white	5	7	8
Orange rave×F_1 intermediate	white	3	8	30
Orange race×F_1 intermediate	white	3	8	30
Orange race×F_1 intermediate	5 *w*♀♀ total	62	69	267
Orange race×F_1 intermediate	white	39	0	33
F_1 inter.×F_1 inter.	white	3	3	13
F_1 inter.×F_1 inter.	intermediate	0	6	6
F_1 inter.×F_1 inter.	intermediate	0	12	22
F_2 inter.×F_2 inter.	intermediate	0	8	14
Yellow race×Orange race	yellow	0	40	122
Yellow race×Orange race	yellow	7	9	45
F_1 inter.×Yellow race	intermediate	0	36	24
Yellow race×grade 6 inter.	yellow	0	4	4
Grade 4 inter.×?♂	intermediate	0	6	9
Grade 6 inter.×?♂	intermediate	0	13	8
Grade ?×?	white	13	53	80

record of their description available. The living males from Rio Hondo, California, as well as a white female sister had blue-green eyes, bluish blood, bluish larvae and pupae.

History of the white male brood is given in table 11.

The white female in B1 died soon after emergence from the pupa without expanding its wings. The males lived for three and four days but could not be induced to mate. In none of these did the proboscis parts unite at the end quarter. Their wings did not harden properly after emergence from the pupal case and were quite abnormal. It was intended to intercross orange brothers

TABLE 11

Pedigree involving the white male strain. The original female was from Rio Hondo, Calif., Feb. 2, 1941.

	WHITE ♀♀	ORANGE ♀♀	WHITE ♂♂	ORANGE ♂♂
A. WILD ORANGE ♀	—	33	—	47
All crosses form A				
B1 Orange ♀ × orange ♂	1	6	2	19
B2 Orange ♀ × orange ♂	—	26	—	27
B3 Orange ♀ × orange ♂	—	8	—	14
Crosses from B3				
C1 Orange ♀ × orange ♂	—	21	—	18

and sisters of these white individuals (B1 progeny), but fumigation of the greenhouse at this time killed all the material.

It seems clear that the gene giving this white male and female brood is not dominant but recessive and different from that gene discussed as a dominant sex-limited one. It may be surmised that the wild orange female (A) or its mate was heterozygous for the recessive gene. Had the male been homozygous for it, (white) broods B2 and B3 would have shown homozygotes as well as B1. Half the individuals of A progeny (33 ♀ 47 ♂) should have been heterozygous for the recessive gene. One-fourth of the broods from these individuals, therefore, should have produced homozygotes. One out of three did; however, considering the lethality of the homozygotes, it is possible that B3 might have had homozygotes which did not reach the late pupal stage when they could be distinguished. The cross from B3 progeny shows that some individuals did not carry the recessive gene for white.

EGG FERTILITY IN VARIOUS CROSSES

Counts on egg fertility were made in hybrid crosses (yellow race × orange race) and in crosses in which the homozygous dominant white might be expected.

The former were made in order to determine whether or not the interracial segregation of genes has led to any lowering of viability, or to the elimination of any portion of the resulting segregants at this time. The latter were made in order to determine if the expected homozygous lethal dominant would show up in the results by a higher than normal death rate in the egg. The counts were not carried on through the larval stages because of the high normal mortality at this time which could not be controlled.

The classification into fertile versus non-fertile eggs was made by direct observation. Fertile eggs will turn red in about one day at 25–28°C; non-fertile eggs will remain the newly-laid color of white or yellow for several days before collapsing. The counts were made on the day following time of laying—always 20–30 hours later. This is ample time for the development of all fertile eggs but yet too short a time for larval emergence. All eggs received identical treatment.

They were laid on alfalfa leaves at 26–28°C, 80 percent relative humidity, and remained at these conditions for one day. Therefore, the effects of normal desiccation should be the same on all eggs.

As a basis for comparison of the various egg fertilities, counts were made of eggs from normal orange and white females. These are shown in table 12.

TABLE 12

The comparative fertility of eggs from wild females. The ratios are given as an indication of whether or not homozygous dominants are expected. The ratio under Westley wild eggs is the ratio of adult females in the population, not that derived from the eggs.

SOURCE OF THE EGGS	RATIO, w ♀ : or ♀	% INFERTILE	N
Westley, Calif., orange ♀	21:18	2.6±0.7	502
Westley, Calif., white ♀ (2–2)	28: 0	8.6±2.2	151
Westley, Calif., white ♀	14:10	4.4±1.5	180
Westley, Calif., wild eggs (random)	74:26	1.1±0.4	662
Elsah, Illinois, orange ♀	0:20	2.0±0.5	792
Elsah, Illinois, orange ♀		1.0±1.9	294
Elsah, Illinois, white ♀ (5–5)	18:21	1.3±0.4	761
Round Valley, Calif. white ♀ (1–1)	6: 0	2.2±0.8	361
Total wild females and eggs, orange race		1.9±0.2	3,703
Round Valley, Calif. y♀ × y♂		9.5±	42
Round Valley, Calif. y♀ × y♂		1.7±	180
Total wild females, yellow race		3.2±3.2	222

TABLE 13

Fertility in the eggs of different white females mated to the same male. See pedigree in table 7.

SOURCE OF THE EGGS	GENOTYPE	% INFERTILE	N
A white ♀ (2-2)*	heterozygote	—	—
B white ♀ (1-1)	?	0.0	19
C white ♀ (3-3)	homozygote	0.9±	907
D white ♀ (5-3)	?	2.3±	44
E white ♀ (5-5)	heterozygote	0.6	1,194
F white ♀ (5-5)	heterozygote	1.9	537
G white ♀ (2-1)	homozygote	2.4	330
H orange ♀ (6-6)	homozygous rec.	2.4	783
I orange ♀ (2-1)	?	1.6	386
Total for all females by same male		1.4±0.2	4,200

* The percentage of fertile eggs from this cross dropped daily from 15 to 100 percent infertile in gradual degrees within five days. On the eighth day, the female was remated to another male and produced 39 fertile to no infertile egg. Presumably she had not enough sperm from the first mating.

The results indicate that two percent or less is the normal frequency of infertile eggs laid. The high infertility (8.6 percent) of the eggs in which homozy-

gotes might be expected (Westley 28:0) is probably not significant. Despite the expected high frequency of homozygotes in the Westley wild eggs (24 percent), there is no reduction in egg fertility (1.1 percent). The eggs from Illinois females with no homozygotes expected give the same results as the California material.

Within the yellow-race, egg fertility counts were obtained on two females only. The results are not essentially different from the orange race.

The crosses made to test the genotype of the variant white females (see pedigree, table 7) yielded the data in table 13.

There is no essential difference between the results whether the female is white or orange or whether the female was a homozygote or heterozygote dominant. Since the male was a heterozygote, all crosses with white should have produced homozygous dominant eggs, one-fourth with heterozygotes and one-half with homozygotes.

Results from another pedigree (table 14) are similar.

TABLE 14

Egg fertility in crosses giving aberrant ratios of white females and also showing a possible difference in inbred versus outbred crosses. The matings are all by a single heterozygous male (pedigree in table 8).

SOURCE OF THE EGGS	GENOTYPE	% INFERTILE	*N*
D_2 white ♀	?	0.6	710
D_2 white ♀	?	2.0	494
D_5 white ♀	heterozygote	1.9	592
D8 white ♀	?	0.7	451
D_9 white ♀	?	0.2	419
Total of the inbred females		0.9±0.2	2,666
D6 orange ♀	homozygous rec.	2.7	866
D_7 white ♀	homozygous dom.	3.6	837
Total of the outbred females		3.1±0.4	1,703

The higher infertility of D_7 eggs compared with the other white females may be related to the outcrossing since its orange sister has the second highest rate. The difference between the inbred and outbred crosses is significant, but the meaning of this difference is unknown.

A lowering of viability might be expected in crosses between the two complexes—orange-race and yellow-race. Though the sterility involved between these is most probably caused primarily by deficient diet, new and unsatisfactory genic recombinations are anticipated which would lower the viability of the subsequent generations. The counts in table 15 were made to test this hypothesis, even though it is expected that mortality will probably occur at later stages as well (that is, larvae and pupae).

These results all show a significantly higher rate of infertility —even as far removed as the backcross. Sisters of the females used in the two backcrosses

TABLE 15

The frequency of infertility in eggs involving intermediate segregations between the orange and yellow races.

SOURCE OF THE EGGS	GENOTYPE	% INFERTILE	*N*
From Round Valley, Calif.:			
Grade 1 ♀ × Grade 10 ♂	F_1 hybrid	22.4	49
Grade 1 ♀ × Grade 6 ♂	backcross	2.9	345
Grade 4 ♀ × ? ♂	prob. backcross	9.1	619
Grade 6 ♀ × ? ♂	prob. backcross	7.4	108
Grade 6 ♀ × ? ♂	prob. backcross	3.3	423
Total F_1 and backcrosses (Round Valley stock)		11.5±0.9	1,154
Sisters of D_1–D_5, D_8–D_9 ♀ ♀ (table 14) in the following:			
White ♀ × Grade 5 F_1 hybrid ♂	backcross	5.7	697
White ♀ × Grade 6 F_1 hybrid ♂	backcross	10.1	248
Total of these two backcrosses		6.8±0.8	935

to F_1 hybrids were crossed to brothers with the following comparison: to brothers—0.9 percent infertility and backcross—6.8 percent infertility.

The F_1 cross with 22.4 percent infertility in the egg was accompanied by aberrant development as judged by the disturbance in red pigmentation of the egg. The disturbances are such as to cause various portions only of the egg to be pigmented or for the pigment to be lighter in color than normal. Cases of this sort have been noted in the eggs of many females obtained at Mono Lake and Round Valley, California, where the yellow and orange races hybridize. Actual counts show disturbances much as in this cross: full red eggs (normal), 26; full orange (lightly pigmented), 4; top portion only red, 5; lower portion only red, 1; tinge of orange only, 2; white, 11.

The yellow female was bred on red clover and the orange male on alfalfa. Therefore, diet should have had no influence on the result.

It is clear that considerable disturbance of development occurs in the interracial crosses together with the lowered egg fertility

GENERAL SUMMARY AND CONCLUSIONS

The genetic difference between the orange and yellow races of *Colias chrysotheme* is a multiple factor one, as shown by interracial crosses. Hybrid individuals are usually fertile.

The two races have a different larval diet necessity even though females of each have been recorded as laying eggs on nearly the same species of food plants.

Sterility, sexual apathy, high mortality, and phenotypic alterations are the result of interchanging the normal food of the races.

The yellow race is able to go into diapause during the middle of the larval stage; the orange race is not able to do so.

The food necessity, diapause, and color differences between the races are genetically controlled but are not interchangeable between the races. However, yellow "orange-race" butterflies do occur rarely in pure orange-race populations.

Intermediates between the races are found in the wild. Genetic results indicate that these may be F_1, F_2, F_n, or backcross individuals. They are fertile. Copulating pairs of many combinations have been taken in the wild.

The white female forms occurring in wild populations of both races are genetically controlled by a dominant allele (confirmation of GEROULD 1923). The homozygous dominant is not distinctly separable phenotypically from the heterozygote, though there is a suggestion that it might be represented by more extremely white individuals. All three expected genotypes occur in wild populations and have been bred in the laboratory.

The homozygous dominants seem to be lethal or semi-lethal with certain modifier environments; this hypothesis supersedes that of GEROULD. There is also a possibility that the homozygous recessive may also be at a disadvantage with certain modifiers. These modifiers are probably selected by the physical factors of the environment in the wild.

The dominant gene for white is readily and easily transferred from one race to the other (confirmation of GEROULD 1923).

The white males known in wild populations are not genetically controlled by the same dominant factor as the usual white females but by a nearly lethal recessive gene. They also have additional phenotypic alterations. White males are found in both races.

The normal egg fertility of Colias is approximately 97–99 percent. The presence of the homozygous dominant white females does not affect this figure.

There is a lowered egg fertility in the interracial crosses and in the ensuing crosses between intermediates and backcrosses. This is partially accompanied by aberrant egg development.

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THE DISTRIBUTION OF GENE FREQUENCIES IN WILD POPULATIONS OF COLIAS

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NEARLY all butterflies of the genus Colias have dimorphic female forms—the normal yellow or orange and the "albinic" or white form. The white form in both races of *Colias chrysotheme* in North America is controlled by a dominant autosomal gene which has effect only on the female sex (GEROULD 1923; HOVANITZ 1943a). This seems to be true also for *Colias myrmidone* and *edusa* (=*crocea*) of Europe and the *christina* complex of North America (GEROULD 1923; FORD 1937; HOVANITZ 1943a). The general distribution and abundance of the white female form of *chrysotheme* in North America have been described in detail (HOVANITZ 1943b).

Owing to the ease of identification of the normal and white forms, this material is excellent for the study of gene frequency variations and its biological causes in wild populations. For this purpose, samples of the populations of the orange race were obtained seasonally and geographically in 1941–1942.

METHOD

Counts of normal and white females were made throughout the alfalfa growing districts of central and southern California, southwestern Arizona, and the western Great Basin. The earliest samples obtained were by actually capturing individuals; later, however, counts alone of free-flying individuals were found to give larger numbers and more significant results.

Knowledge of the exact viability of the three genotypes expected in the populations in all locations is necessary before pertinent gene frequency data can be calculated according to the standard formula: $p^2+2pq+q^2$ (HARDY 1908). Since the experimental data suggest that the viability of the dominant homozygote (q^2) may be zero under certain conditions and equal to or better than that of the other genotypes under other conditions, the application of this formula under known conditions of viability is not possible. The data are therefore discussed not as gene frequencies but as phenotype frequencies only.

With no difference in viability between the genotypes, the gene frequency in a population will remain constant, with random fluctuations only (WRIGHT 1932, 1942). With complete lethality of the dominant homozygote, which is probably true in hot environments and in southern regions, the white allele will be eliminated at a rate as given in table 1.

The white allele probably has a selective value under certain environmental conditions which is greater than that of the normal yellow or orange allele. According to the conclusions derived from museum data alone (HOVANITZ 1943b), the white allele should be at an advantage over the normal allele in the north as compared with the south. Therefore, in this detailed analysis of populations, a similar north-south rule should be found. The allelic frequency

should vary directly with the change in climate between regions if the two are thus interrelated. In a previous paper (HOVANITZ 1942), it was stated that only by a polygenic or multiple allelic hereditary mechanism can a gradient of morphological, adaptive variation coincide with a gradient of changing environmental conditions. When this kind of hereditary system is not available, similar results may be achieved by varying ratios of two alleles with extremes of 100:0 and 0:100.

TABLE 1

Table showing rate of elimination from a population of a dominant allele which is homozygous lethal, population size being at infinity. Assuming equal viability for the other two genotypes, the elimination will be at the rate shown by the decrease per generation.

FREQUENCY OF WHITE ♀ ♀, Ww	DOMINANT ALLELE FREQUENCY, W	GENERATION
66.7%	33.3%	1
50.0%	25.0%	2
33.3%	16.7%	3
28.6%	14.3%	4
25.0%	12.5%	5
22.2%	11.1%	6
20.0%	10.0%	7
18.2%	9.1%	8
16.5%	8.3%	9
15.4%	7.7%	10
9.1%	4.6%	20
1.96%	.98%	100
.199%	.099%	1000
.000199%	.000099%	1,000,000

The data presented here illustrate the correlation between the gradient formed by the frequency of the two white alleles and the gradient formed by the climatic conditions of the territory. They also show the effects of migration and random population change upon the gene frequency in various environments. A differential development rate of different genotypes and its effect upon population differences is noted in two populations.

THE FREQUENCIES OF THE WHITE FEMALE IN CALIFORNIA POPULATIONS

The frequencies of white females in California populations of the orange race are shown on the map (fig. 1). The general trend of highest frequencies in the north (69 percent white) and lowest frequencies in the south (13 percent white) is apparent.

The region sampled is divided into five distinct provinces by the local topography of the land. There are from north to south: (1) the San Francisco Bay area in the northwest; (2) the San Joaquin Valley area along the central portion of the state; (3) the Great Basin, represented on the map by Mono Lake and Round Valley; (4) Coastal Southern California, represented by scattered alfalfa fields throughout the coastal valleys; (5) Coachella-Imperial-Colorado River Valley area in the southeast.

The genetic nature of wild populations are studied or visualized only with the aid of maps, climatological information, and a knowledge of the local topography.

To neglect these data would be to eliminate the causal factors in the population differentiation; the cause of the differentiation is the point of this study.

FIG. 1.—Map showing the locations in California and southwestern Arizona of the populations sampled for the white gene frequency and population structure of the races of *Colias chrysotheme.*

The population differences (seasonal and geographical) are described below, starting with a central point, the coastal Southern California populations.

THE COASTAL SOUTHERN CALIFORNIA POPULATIONS

The Coastal Southern California populations sampled are shown on the map (fig. 1) by numbers derived from the white frequency at each place.

The San Gabriel Valley area

The localities known collectively as the San Gabriel Valley area are situated at the lower end of the valley in the vicinity of El Monte, Garvey, and Puente. The locality is marked on the map (fig. 1) by the number 30. The populations

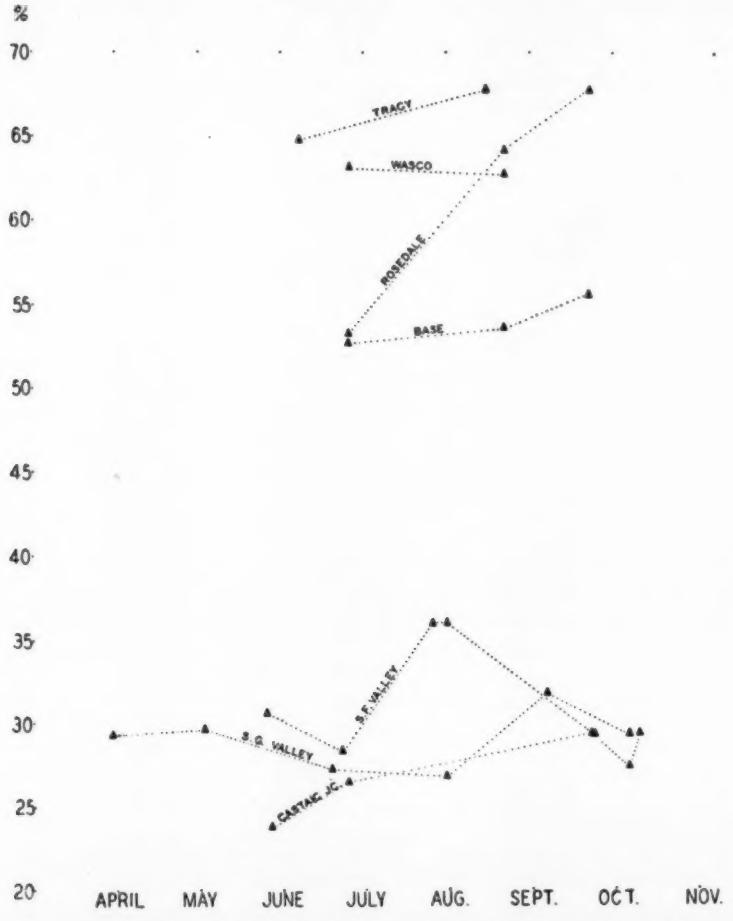

FIG. 2.—Showing the frequencies of white females as compared with orange during the seasons at various localities in California. Compare these minor fluctuations with the large ones of Mono Lake (fig. 6).

are split into two parts, separated by the San Gabriel River, the centers of which are about five miles apart. Unless specified, samples were obtained generally over the area.

The general trend of the frequency variations during the 1942 season is given on the graph (fig. 2) as "S. G. Valley." It is seen that there is little fluctuation during the season. A summary of the data according to the season for 1941–1942 is given in table 2. There are no significant differences in any of the

TABLE 2

Seasonal frequency of white females in the San Gabriel Valley for two years.

SEASON	% WHITE ♀♀	N
1941		
Spring	21.62±6.77	37
Autumn	30.03±1.45	999
Total	29.73±1.42	1,036
1942		
Spring	33.33±7.27	42
Early summer	28.15±1.55	906
Late summer	33.37±1.65	812
Autumn	28.75±1.82	612
Total	30.19±0.94	2,372
Grand Total	30.05±0.78	3,408

seasons, nor between the two years 1941 and 1942. Were the population number reduced to an exceedingly low value, it might be expected that random changes in gene frequency would occur, but no such variations are present. Apparently the population size is very large, or the environmental selection for the white and orange alleles is rather closely regulated. Both conditions probably prevail.

In May and June, 1942, separate data were obtained on each side of the San Gabriel River (table 3) to determine if a difference might exist, due to the spatial isolation. This is a test of degree of population movement as well as of size, since a difference in the results would indicate that the area is not well mixed or that random changes have taken place in gene frequency.

TABLE 3

Frequency of white females at two localities on the two sides of the San Gabriel River (in the San Gabriel Valley). 1942. The numbers show the percentage of white females.

	WEST SIDE	N	EAST SIDE	N	TOTAL	N
May	27.27±4.48	99	30.57±3.21	229	29.51±2.51	328
June	25.40±2.10	433	33.10±3.91	145	27.34±1.83	578
Total	25.75±1.89	532	31.55±2.40	374	28.15±1.55	906

In both May and June, the frequency was lower on the west side than the east side. In neither case was the difference equal to or greater than twice the standard error. With the combined data, the difference is 5.80±4.54 percent or considerably less than twice the standard error of the difference.

The San Fernando Valley Populations

The San Fernando Valley populations are located about 30 miles northwest of the San Gabriel Valley. On the map (fig. 1) the place is denoted by the number 30. Between these two localities is located the city of Los Angeles and two "mountain ranges" (Santa Monica mountains and part of the "Puente Hills"). Therefore, there are no alfalfa fields to unite the two places. The chance of much gene exchange across this 30 miles under normal circumstances would seem rather doubtful. However, the species is somewhat migratory, and at times there is probably considerable exchange. The climate at the two places is very similar, being under the same influence of the prevailing westerly winds and ocean fog. There should be very little differential climatic selection.

TABLE 4

White female frequency during the season at San Fernando Valley, Calif. 1942.

DATE	% WHITE ♀♀	N
May 25	30.57±3.04	229
June 22	28.43±2.01	503
July 25	35.97±4.06	139
Aug. 1	35.96±4.50	114
Sept. 7	17.95±6.15	39
May–Sept. 7	30.37±1.43	1,024
Sept. 23–Nov. 7 (see table 5)	30.29±0.43	11,149
Total	30.30±0.42	12,173

The trend of the variations in this region is shown on the graph (fig. 2) as "S. F. Valley." The variations in the populations of the San Fernando Valley are not statistically different. Even the July and August percentages of 35 percent white are not different from the average of 31.48 for this period (table 4).

During the period September 23 to November 7, tests were made to determine if there were a differential development rate between the two types of female. One particular field in the San Fernando Valley had a heavy infestation of Colias and was in full eclosion. Frequencies were obtained in this field at two day intervals of (a) percentage of white females freshly eclosed on that day and (b) percentage of white females free-flying in the population. Frequencies were obtained in local fields nearby to serve as a control.

The graph (fig. 3) shows the variation in frequencies between newly emerged females in one alfalfa field (as judged by the fact that they had wet wings and were in copulation) and the frequency of free-flying females in the same place. There is also shown by dotted lines the frequencies in two control fields about one mile from the former place.

From a high of 36 percent white females which eclosed on September 23, the frequency dropped to 29 percent on each of the two succeeding periods of

two days each to a low of 24 percent on September 29. The difference between the extremes is 11.87 ± 4.23 percent or nearly three times the standard error of the difference (table 5).

Two days following the sample of 36 percent of newly emerged females, samples were made of the frequency of free-flying individuals. On September 25, the frequency was 45 percent. This dropped at a rapid rate at first but was slower later until a low of 25 percent was reached on October 5. The difference between these extremes is 19.35 ± 2.50 percent, or nearly eight times the stand-

TABLE 5

The frequency of white females at a given alfalfa field in the San Fernando Valley during the course of an adult eclosion period. The copulated females were newly eclosed on the day of the sample The control samples were made in fields approximately one mile from the tested field.

DATE	FREE-FLYING %w	N	COPULATED, %w	N	CONTROLS, %w	N
Sept. 23	—	—	35.91 ± 2.96	323	29.24 ± 1.96	537
Sept. 25	44.58 ± 2.04	590	29.22 ± 2.36	373	32.22 ± 2.58	329
Sept. 27	37.61 ± 2.07	561	29.20 ± 3.06	226	27.81 ± 2.31	374
Sept. 29	32.85 ± 1.85	685	24.04 ± 3.01	208	25.12 ± 2.17	414
Oct. 1	29.59 ± 1.59	828	—	16	29.45 ± 2.52	326
					$(31.05 \pm 1.96$	554)
Oct. 3	27.75 ± 1.76	645	—		$(28.61 \pm 2.29$	423)
Oct. 5	25.23 ± 1.46	880	—		$(27.40 \pm 1.99$	500)
Oct. 7	29.97 ± 1.47	971	—		$(30.08 \pm 2.40$	359)
Oct. 9	26.90 ± 1.78	617	—		$(29.49 \pm 2.57$	312)
(Nov. 7)	$(37.76 \pm 4.88$	98)	—		—	
Total	31.30 ± 0.61	5875	29.76 ± 1.35	1146	29.00 ± 0.71	4128

ard error of the difference. Some of the consecutive samples are likewise more than twice the standard error of the difference. For example, that between September 25 and 27 is 6.97 ± 2.93 percent, or over two times. The difference between September 27 and 29 is 4.76 ± 2.78 percent, or just under two times.

On October 7 and 9, the population seems to have leveled off to a standard range of fluctuation, and after the ninth, individuals were scarce. As far as adults were concerned, the population size was greatest between September 23 and 27, when the alfalfa field was literally covered with them. At this period all individuals were exceedingly fresh. From September 27 to October 9, the population size decreased rapidly. Samples of newly emerged adults could not be obtained after September 29 owing to the very rapid drop in eclosions. By October 3, fresh individuals were rare, and by the ninth nearly all were old and worn. The alfalfa field was being cut from about four days before September 23 and was completely cut on the twenty-seventh. At this period thousands of pupae were crushed. By October 9, the shortest average new growth in the field was about one foot high; females rarely lay eggs in a field higher than this.

The control samples were gotten in two fields about one mile east of the

field being tested. The fluctuations in the control samples are not statistically different but they follow the experimental curves to a certain extent. There is a drop between September 25 and 29 following the drops in the other curves; also a drop followed by a rise between October 1 and 9 following the same in the experimental curve. The control plots were in fields which did not have a high percentage of eclosing individuals. Their population was at least largely

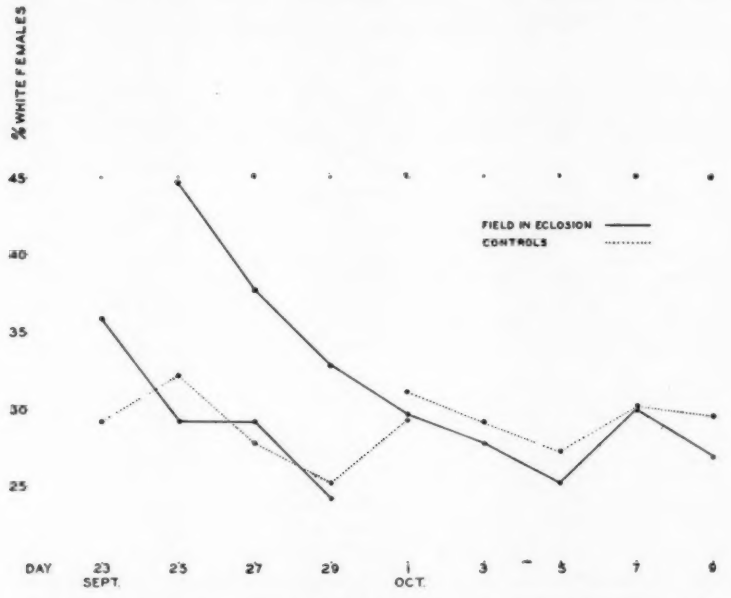

FIG. 3.—Showing the frequency of white females in a San Fernando Valley (California) population during an adult eclosion period (upper heavy line). The lower heavy line represents freshly emerged females with wet wings. The dotted lines represent control samples in other alfalfa fields. The first control was replaced by a second on October 1.

composed of individuals which had flown in from elsewhere. This was especially true of the field used between September 23 and 29. The controls are more representative of the valley populations as a whole than the experimental population. Two possibilities may account for the very rapid gene frequency change in the one field: (a) the white individuals are faster in development than the orange so that there are more of them at the earlier stages of the adult eclosion, or (b) the white gene has somehow gotten a rather high concentration in one place due to chance.

The former possibility seems more likely, because it fits all the data better. One can account for the higher frequency in the beginning in both experimental curves by assuming that more white females emerge at first. Since more orange females would emerge late, it would be expected that the frequency of white

females would drop below the average for the region, provided that there was a general exodus of adults from the area. The assumption of decrease in adults is correct, because (a) the number of adults visibly declined from September 27 on, (b) adults were seen to be flying away from the field in large numbers, (c) the area already had a higher concentration of adults than any other place in the valley; therefore, more adults would leave than arrive, and (d) a percentage of the early emergents would have died, thus lowering the number of adults there. As expected, the frequency of whites dropped below the average frequency of 30 percent to a low of 25 percent. In favor of the first possibility also is the drop in frequency in the newly emerged females.

The tendency of the control curves to follow the experimental ones can also be explained by the first possibility (a). Since adults were leaving the field and scattering in all directions, it may be assumed that the higher concentration of whites at the one place may influence those nearby.

On the second possibility, one must assume that a very rare chance concentration of white females had laid the eggs from which the brood arose. The number of females must have been huge considering the size of the brood. The larger the number, the less the chance of getting a concentration fluctuating from the normal. The size of the brood must have been in the millions.

On the basis of the second possibility, there is no way of explaining the drop in frequency in the newly emerged insects unless one assumes that one day a month before the emergence mostly white females had laid eggs in the field and a few days later a higher frequency of orange females laid eggs. The likelihood of this being true seems nil.

The second possibility assumes that the drop in frequency is due to a mixing of the adults with the general valley population. This would entail a great movement of adults into the field. On the contrary, the movement was visibly away from the field. The frequency would not have dropped below the general valley frequency of 30 percent by this method, nor would it have dropped so quickly.

Therefore, unless the development rate of white females is faster than that of the orange, it does not seem likely that the change in gene frequency could have occurred. This conclusion is in agreement with a similar conclusion reached on the basis of breeding data.

Populations of the Santa Clara River Drainage Basin

Airline about 15 miles north of San Fernando Valley along the central part of the Santa Clara River Valley, three samples were made during 1942. This population (Castaic Junction) is located on the map (fig. 1) by the number 27. The fluctuation during the season is not significant but varied from 25 percent white in May to 27 percent white in June to 29 percent white in September. This gradual rise is illustrated on the graph (fig. 2) as "Castaic Jc." It is possible that the frequency has gradually risen during the season, and it could easily have done so by migrations from the extensive populations in the San Fernando Valley. There is, however, a range of hills separating the two places (Santa Susana Hills). Comparing these two localities for the 1942 totals shows

that "Castaic Junction" has a frequency which is statistically lower than that in the San Fernando Valley:

		N
Castaic Junction	27.44±1.38 percent	1,046
San Fernando Valley	30.30±0.43 percent	12,173
Difference	2.86±1.44 percent	

"Castaic Junction" is farther north than San Fernando Valley but has a much hotter and dryer climate owing to its sheltered location within the hills and far from the coast. The populations in the San Fernando Valley are at the westernmost and coolest portion of the valley. The inversion of the north-south "rule" in this case may be due to the climatic regularity or possibly to chance alone.

Down the Santa Clara River Valley from Castaic Junction toward the coast is a distance of 40 miles airline. Apparently there are few alfalfa fields between these two places and not very many even at the coast. However, a small sample was gotten at the latter place (Oxnard) with a frequency of 43.75±6.20 percent white females (see map 1 at point marked 44). Climatically, the area is cooler, foggier, and more humid than any of the other Southern California locations. It should have a higher frequency of whites, as is indicated. However, the small number renders the significance of the data doubtful. The frequency is apparently statistically different from the two closest populations by more than twice the standard error of the difference: Difference from Castaic Jc. is 16.31±6.35 percent and from San Fernando Valley is 13.45±6.40 percent.

In one of the branches of the Santa Clara River Valley known as Bouquet Cañon, a sample was gotten in May 1942. This place is about ten miles east of Castaic Junction and is more or less connected with it by occasional alfalfa fields. The frequency found here in May 1942 was 39 percent. The number of individuals again was low, and the frequency, therefore, is once again doubtful. Compared with the populations at Castaic Junction, the difference is nearly four times the standard error of the difference for the month of May and about five times for the seasonal average at the latter place:

		N
Bouquet Cañon	39.34±6.25 percent	64
Castaic Jc. May	23.64±4.05 percent	110
Difference	15.70±4.55 percent	
Castaic Jc. 1942	27.44±1.38 percent	1,046
Difference	11.90±2.45 percent	

The frequency of 39 percent is very close to the frequencies in the Antelope Valley to be considered later and may owe its origin to that source.

The Antelope Valley Populations

The Antelope Valley is located about 25 miles east of the Bouquet Cañon population. It is really the western arm of the Mojave Desert but has considerable agriculture due to the presence of water for irrigation. The northern arm of the San Gabriel Mountains extends between the desert and the coast here, but the elevation is very low, owing to the change in geological structure. Cold winds from the coast blow through the area causing it to be climatically quite different from other parts of the desert. In the summer it may be very hot or may be cold and windy; in the winter it is colder than the Southern California coast.

Samples were obtained in May and June at two places, Palmdale and Lancaster. Between these localities, a distance of eight miles, there is no alfalfa, so that the places may be considered as isolated by dry desert. At Palmdale in May the frequency was 39.44 ± 4.1 percent ($N = 142$). In June it was 31.96 ± 2.72 percent ($N = 291$). In June at Lancaster the frequency was 38.83 ± 4.81 percent ($N = 103$). None of these differences approaches statistical significance. The total for Antelope Valley for the season, 35.26 ± 2.08 per cent ($N = 536$) is not different from the closest population, Bouquet Cañon.

At first sight it might seem that the desert should have a low frequency of the white gene. However, this part of the desert is cold in winter and does not always get hot in summer. In 1941, cold winds were almost continuous throughout the summer with only one or two "warm spells."

The Antelope Valley populations are the stepping stones to the San Joaquin Valley populations farther north with frequencies above 50 percent. The more coastal populations of Southern California are separated from the San Joaquin Valley by 70 airline miles of rugged mountains, while Antelope Valley is separated from it by only 20 miles airline across the range. The map does not illustrate the difference, since the Lancaster-Palmdale areas are far to the southeast in the Antelope Valley.

Other Southern California Populations

Samples were obtained at four other coastal localities which are designated on the map (fig. 1) by the white female frequency—namely, Santa Ana, 29 percent, San Pasqual-Escondido, 18 percent, San Luis Rey, 25 percent and San Jacinto Valley, 23 percent.

The Santa Ana population on the map is 20 miles airline directly south of the San Gabriel Valley samples. The area in between is nearly continuously strewn with alfalfa fields in the lower places. The climate is probably a little cooler in the summer and warmer in the winter, since it is closer to the coast; however, this is only very slight. The white female frequency is identical with that of the San Gabriel Valley.

The San Jacinto Valley population is about 50 miles airline due east of Santa Ana (number 23 on fig. 1). Owing to its inland location and higher elevation of 2,000 feet, it has a much warmer and dryer climate in the summer and colder in the winter. The white female frequency is lower here, as is expected con-

sidering its climate and location. The significance of the difference between its frequency and adjacent populations follows:

		N
San Jacinto Valley	22.65±1.82 percent	521
Santa Ana	29.41±2.02 percent	510
Difference	6.76±2.71 percent	
San Pasqual-Escondido	18:47±2.19 percent	314
Difference	4.18±2.84 percent	
Thermal	12.95±2.85 percent	139
Difference	9.70±3.78 percent	

The San Jacinto Valley-Santa Ana difference is just over twice the standard error of the difference. The difference with San Pasqual-Escondido is nearly twice while the difference with Thermal is almost three times.

The San Luis Rey frequency (25 percent) is so much like others in the vicinity and the number in the sample is so low that it is hardly different from any adjacent population. The place is located on the coast about 50 miles southeast of Santa Ana. The climate is very similar to that place.

The San Pasqual-Escondido population is located inland about 15 miles southeastward of San Luis Rey. The climate is likely to be warmer than the latter place or Santa Ana and quite similar to San Jacinto Valley. We have seen that the frequency is about twice the standard error of the difference in comparison with the latter place. It is close to or identical with the frequencies in the Imperial-Coachella Valley and quite different from Santa Ana and San Gabriel Valleys. On the coastal side of the mountains, it is the lowest frequency known and is likewise the most southern known.

THE COACHELLA-IMPERIAL-COLORADO RIVER VALLEY POPULATIONS

The Coachella-Imperial-Colorado River Valley is relatively isolated from the other populations considered before by the mountain ranges which include the San Jacinto range, the Santa Rosa range, and the Laguna-Cuyamaca mountains. This region is one of exceptionally low rainfall, receiving less than five inches annually. It must have been completely uninhabited by *Colias chrysotheme* before the period of irrigation, due to the lack of any larval food. The valley floor was covered only by shrubs typical of the Coachella and Colorado deserts before irrigation. The area is largely below sea level, and all samples aside from Yuma were obtained below the level of the sea. At Kane Springs, the elevation is about −200 feet. A few alfalfa fields exist north of the Salton Sea, at which place the sample at Thermal was obtained. Between Thermal and Kane Springs the area is almost all barren desert rock or sand and the water surface of the Salton Sea; this is a distance of about 50 miles. From Kane Springs to Calexico on the Mexican border the area is commonly

continuously covered with irrigated alfalfa fields. Climatic conditions are mild in winter so that the populations can be breeding all year. In summer, the temperatures rise to 130°F at times; occasionally, for periods of one or two weeks constantly the temperatures day and night will not drop below 100°F. Such continuous temperatures are sufficient to sterilize the butterflies of the coastal population (at least with a high humidity). Possibly there has been established a physiological race able to withstand such conditions, or perhaps the low humidity of the region allows cooling by evaporation.

The white female frequency in this area is the lowest known, varying from 12.9 to 17.8 percent. The differences in the populations to be described are hardly to be correlated with climatic differences, though such correlations are yet to be made accurately. The valley is climatically very similar from the Mexican border at least to Indio. Farther north, cooling by winds from the San Gorgonio Pass takes place.

The frequency at Thermal, 12.95 ± 2.85 percent is different by nearly three times the standard error of the difference from the nearest "coastal" population at the San Jacinto Valley. They are separated by a mountainous area about 50 miles airline distance in which there are no alfalfa fields. The butterflies breed in the mountain meadows in the summer, however. The next nearest population sampled is at Kane Springs which is not significantly different from it: 17.80 ± 1.49 percent ($N = 663$); difference $= 4.85 \pm 3.57$ percent.

The Kane Springs frequency of 17.8 percent is obviously not different from the next population about 15 miles south, Brawley, 14.48 ± 2.06 percent ($N = 290$). Brawley in turn is not different from Calexico about 20 miles farther south, 12.99 ± 1.57 percent ($N = 462$). The extremes, Kane Springs and Calexico, are different by more than twice the standard error of the difference (4.81 ± 2.16 percent).

It should be noted that there are almost continuous alfalfa fields from one place to the other and that the distance is but 35 miles.

The average for the Coachella-Imperial Valley is 15.32 ± 0.91 percent ($N = 1554$), which is obviously very different from the average for all of coastal Southern California (including Antelope Valley) of 29.78 ± 0.32 percent ($N = 19{,}050$).

Along the portion of the lower Colorado River just south of Yuma, Arizona, on the east side of the river, there is a considerable extent of irrigated agricultural area. Alfalfa fields are strewn throughout the area from Yuma to San Luis on the Mexican border. The sample of the Colias population here was gotten in various fields along the 15 mile airline distance rather than from any particular field (fig. 1).

The climatic conditions in this area are the same as in the Imperial Valley, and it would be expected that the white female frequency would be either the same or lower. This place is about 40 miles airline east of Calexico, but this distance is barren, waterless desert of moving sand dunes and partly creosote-bush vegetation. Without flowers for nectar, adults could hardly travel this distance in the dry atmosphere, especially when it is so hot. In the winter mild weather, however, an exchange of adults may take place. An adult female

which was well fed as a larva will live for two or more very active days with no food or water and be capable of laying a hundred or more eggs even with no more food.

The frequency of white females in the Yuma-San Luis area is 19.54 ± 1.91 percent ($N = 481$). Compared with the Imperial Valley at Calexico, this is two and one-half times the standard error of the difference (Diff. $= 6.55 \pm 2.37$ percent).

It was expected to find the frequency in the lower Colorado River area to be lower than the Imperial Valley. However, the approximate frequencies derived fron the museum material (HOVANITZ 1946b) shows that Arizona as a whole has a frequency of about 39 percent white. Nearly all these data are from the portion of Arizona at the higher elevations. Locations closer to the latter region would be expected to have a frequency varying toward it provided there is some population interchange. Migration could easily take place up and down the Gila River, for alfala fields tend to follow it. This river connects the Yuma area with the Phoenix agricultural area where alfalfa is very commonly grown and where the fields are heavily infested with Colias (WILDERMUTH 1914).

Including the Yuma area in the total, the southeastern region on the map (fig. 1) has a frequency of 16.31 ± 0.82 percent white females ($N = 2{,}035$).

THE SAN JOAQUIN VALLEY POPULATIONS

On the map (fig. 1), the San Joaquin Valley lies along the central strip of the state between the places marked North and South San Joaquin Valleys. Actually the San Joaquin Valley is the larger and southern portion of the Great Valley of California which is continuous from the south at the Tehachapi mountains to the Trinity-Cascade ranges in the north. The climate in the San Joaquin Valley is "mild" the year round. Summer days are normally hot (above 100°C) but the nights are cool. Winters are cool but not cold; frost forms for two or three months of the year especially to the north. Rainfall is highest in the north (from ten to 20 inches) and lowest in the south (about five inches or less). Temperatures are higher in the south than in the north, but the humidity is lower in the south than in the north. Alfalfa is grown very extensively in the valley, especially in the region at the northwest (at the big triangle on the map), at scattered areas throughout the central part and very commonly in the southern region near Bakersfield (at the rectangle on the map).

The white female frequency throughout this area is over 50 percent as compared with about 30 percent in southern California. The two zones are separated by a mountain barrier which, however, is, not barren of Colias populations. The individuals are merely more scattered, as they must have been over the entire west before alfalfa was grown. The population size must be relatively small; consequently, the gene frequencies would be controlled primarily by migrations from the adjacent alfalfa fields. The population size in the alfalfa fields of California is so huge at the present day that, except in some very isolated places, the gene frequency is probably controlled by them everywhere.

On the map (fig. 1), the general white female frequencies may be observed, these rising from the southern part of the San Joaquin Valley at 58 percent, to 69 percent in the north.

The South San Joaquin Valley Area

Samples were made in this area at points about six to ten miles apart. These data are too detailed for the large maps, and the area represented thereon by a rectangle is shown enlarged (fig. 4). The rectangle represents an area about 60 miles long in a northwest-southeast direction and about 25 miles wide. Alfalfa

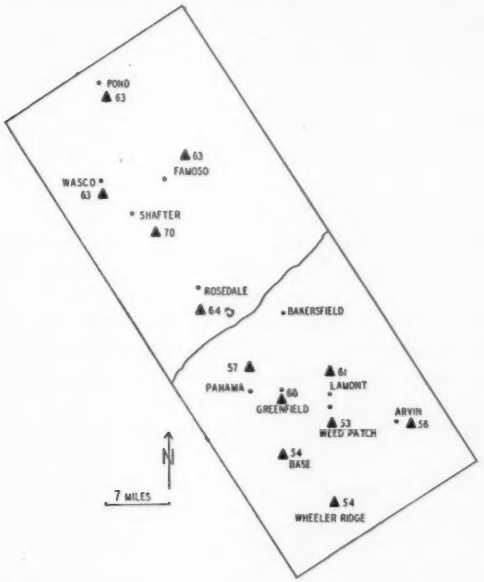

FIG. 4.—The South San Joaquin Valley area, as represented on figure 1 by a rectangle. Dots are locations of towns, and triangles are locations of alfalfa fields sampled. The Kern River is shown by the wavy line through the center.

fields in this area are located in relation to the distribution of the sampled populations. However, there are more fields in the north than the samples would indicate. Near the limits of the rectangle in the southeast and southwest the alfalfa fields are isolated plots surrounded by unirrigated desert. This is true to a certain extent in the central and northern parts, too, but here also there is irrigation of sugar beet farms, beans, etc. Just north of Bakersfield and running southwest between Rosedale and Panama is the Kern River. Its flood basin occupies a wide area in which there are no alfalfa fields. The river itself is no barrier. The area to the north and west of the rectangle is partly dry, unirrigated hardpan soils and partly agricultural. There is no sharp delimitation of the alfalfa-Colias populations in either the west (north of the Kern

River), the north, or the northeast. However, the zone directly north of Bakersfield and east of Famoso and Shafter is largely unirrigated lands left in semi-desert state. Completely around the east side of Bakersfield, Arvin, Weed Patch and toward Wheeler Ridge, the area is devoid of alfalfa. Much of it is devoid of irrigation. West of Base and Wheeler Ridge, alfalfa is occasionally present, but not too commonly. This spotty distribution of breeding places for Colias is of signal importance in understanding the rapid change in gene frequencies which take place.

The frequencies for the San Joaquin Valley placed on the map (fig. 4) are those obtained on August 21, 1942. Three localities were sampled earlier, on June 25, 1942, and two of these later on September 22, 1942.

The three population samples in June are from north to south:

		N
Wasco	63.19±1.08 percent	1,964
Rosedale	53.33±1.31 percent	1,440
Base	52.68±1.28 percent	1,528

Wasco is obviously quite different from either Rosedale or Base. Being in the north, it is expected that it would have a higher frequency than either of the latter two. However, since the distance is small, chance variations within sub-units of a population could be expected to alter the significance of the north-south rule. In climate, Wasco is but slightly if any different from Base or Rosedale. It might have been expected that Rosedale would have had a frequency exactly intermediate between Wasco and Base, since it is geographically located exactly between the latter two (fig. 4). This was not the case, however, for though it was slightly higher than Base, the difference is not significant even with the large sample.

At the later date (August 21), two months later, these same localities were again sampled. The frequency at Base remained essentially the same (fig. 4); the frequency at Wasco also remained the same. Rosedale, however, had risen and was now equal to Wasco rather than Base:

		N
Wasco	62.85±1.57 percent	942
Rosedale	64.16±1.68 percent	812
Base	53.73±2.51 percent	389

The change in gene frequency necessary to alter the phenotype frequency this much is great. The two months between the sampling dates is long enough in the vicinity of Bakersfield at that time of year for two generations. Therefore, sometime about the 20th of July there was a flight of adults which laid the eggs from which came the August 21, adult flight. Possibly at this time there was a movement of adults southward sufficiently to alter the gene frequency. The population size itself is so enormous that the change could not have come through chance. If selection were the causal factor, it, too, would have had to be very great. Assuming equal viability of the three possible genotypes, the gene frequency for a population of 50 percent white would be

33.5 percent dominant alleles and for 66.7 percent would be 44.4 percent dominant alleles. The selection could have occurred against or for any of the three genotypes. Were the homozygote dominant completely lethal, the change from 50 percent to 67 percent could be accomplished in one generation (see table 1), providing selection against the two remaining genotypes was equal. The former probability of mass movement or shifting of the adult population is considered the most likely explanation for the change (fig. 2), especially considering the spotty distribution of breeding places.

The distribution of frequencies in the areas adjacent to these latter three places is a good key to the verification. Arvin, Weed Patch, and Wheeler Ridge are the western and southernmost localities adjacent to Base. They are 56, 53, and 54 percent respectively. North and east of Base are Lamont 61 percent, Greenfield 66 percent, and Panama 57 percent. The latter two are exactly intermediate to Rosedale. The frequency of Panama is closest to Base, but the locality is closest to Rosedale. The reverse is true for Greenfield. It is possible that a flight of adults from the north came through the area a generation earlier and laid eggs in the freshly cut alfalfa fields. Colias apparently migrate more under certain environmental conditions than under others (HOVANITZ 1943d; WILLIAMS and BISHARA 1929), and it is very reasonable to assume such a mass migration at one time but not another. Females normally migrate to cut fields for egg laying. Since the fields are not all cut at the same time in an area, the infestation will be spotty. Thus, places like Greenfield and Rosedale may get an infestation which arose from farther north and which does not belong to the area. Lamont may have gotten a smaller influx.

The localities north of Rosedale all have a frequency above 63 percent; Shafter is highest at 70 percent. The latter is statistically different from all the populations other than Greenfield.

The average for all the localities north of the Kern River (Rosedale and north) is statistically higher than the area south of the Kern River:

		N
North of Kern River	64.50±0.79 percent	6,450
South of Kern River	57.65±0.69 percent	5,237

It seems that the segregation of a small area like that south of the Kern River could not be maintained at lower gene frequency without either a complete isolation with no selection for any allele, or a selection rate for an allele which is at par with the influx of the other allele. There is no isolation except distance between the above two areas. The only reasonably close populations to this area are in the north; the gene frequencies there are all high. The butterflies are known to fly great distances and to move in great quantities. Therefore, there seems to be no alternative other than to assume *selection against a very high concentration of dominant white alleles in the southern area.* This selection for the area must eliminate as many dominant alleles per year as migrate into the region from the north. There is no higher migration of recessive alleles from any direction into the southern area.

The samples obtained from Rosedale and Base in September were very small, for cold weather had killed most of the brood indirectly (through action of parasites on the eggs and young larvae). The graph (fig. 2) shows that the samples were of nearly the same frequency as in August; the slight increase was not significant:

		N
Rosedale (September)	67.65 ± 2.88 percent	272
Base (September)	55.56 ± 4.86 percent	188

Most of the butterflies were very old and worn at this time, suggesting that these were stragglers from the August emergence. It is quite likely, for the September cool weather would not have allowed a new generation but would have allowed the adults to live longer. Eggs laid in August were noted dead on the alfalfa leaves in great quantities.

June and July averages for the South San Joaquin Valley area are as follows. These are hardly comparable, owing to the additional and different populations sampled in August, but they are statistically different:

		N
June	57.06 ± 0.72 percent	4,932
August	60.50 ± 0.46 percent	8,983

The North San Joaquin Valley Area

Samples were made in this area at distances averaging about the same as those in the South San Joaquin Valley area—namely, six to ten miles. The area is depicted on the large map by a triangle (fig. 1). The details of each sample are shown on the enlarged map (fig. 5). The distance along the hypotenuse of the triangle is about 56 miles; each of the other sides is about 35 miles. Most of the localities sampled lie almost in a straight line along the hypotenuse; this compares with the longest length of the South San Joaquin Valley area. One locality lies at the right angle corner and each of the other two are away from it toward the acute angles. Separating the line of seven populations along the hypotenuse from the other three locations is the meandering San Joaquin River and its flood basin. Owing to this break in the valley agricultural area, the distances between the west San Joaquin River populations and the east San Joaquin Valley areas are nearly twice normal.

Alfalfa fields in this triangular area are most abundant along the zone between the San Joaquin River and the Coast Range hills. The hypotenuse of the triangle roughly designates the edge of the hills. The alfalfa fields are less abundant on the east side of the San Joaquin Valley and disappear beyond Ripon. There are few directly along the San Joaquin River. South of Newman and north of "County Line" the fields are still quite numerous. In the Coast Range hills, there is none for many miles.

A sample was made at Tracy on June 7, 1942. All the other samples, including another at Tracy, were made in the two-day period, August 14 to 16, 1942. One population, Westley, was again sampled on the 18th. The frequency in June at Tracy was not very different from that in August, the slight increase not being significant (fig. 2):

Tracy		*N*
June	64.67±2.37 percent	300
August	67.88±1.36 percent	1,180

The August frequencies suggest a concentration of white genes in the region around Westley. From there to the east and north (and also possibly to the south) the frequency decreases. These differences are statistically significant.

FIG. 5.—The North San Joaquin Valley area as represented on figure 1 by a triangle. The wavy line is the San Joaquin River. The small triangles are locations of alfalfa fields sampled, and the dots are towns or landmarks.

The averages for the three central populations, Westley, Vernalis, and Yarmouth, are shown here for comparison with the two northwestern populations, the three eastern ones and the two southeastern ones:

Central		*N*
(Westley, Vernalis, Yarmouth)	72.25±0.86 percent	2,729
Northwest		
Tracy, County Line)	64.85±1.01 percent	2,202
East		
(Manteca, Ripon, Modesto)	66.77±1.07 percent	1,950
Southeast		
(Patterson, Newman)	66.72±1.55 percent	895

The central area is obviously different from each of the others except the southeast. The standard error of the difference of these two is just twice the difference—that is, 3.53 ± 1.78 percent. Neither of the other three areas are different from one another, though some of the individual differences are great. "County Line" has a low of 61 percent, the lowest in the northern populations; it is statistically different from all the rest.

Judging from visual approximation, the population abundance was also highest in the central area, especially at Westley. At Yarmouth and Westley, the population consisted primarily of newly emerging adults; wet winged individuals were common. At the other localities, the individuals were apparently not so fresh. In a freshly cut alfalfa field, the ratio of males to females is a fair indication of the new emergence. A cut field with no new adults eclosing is nearly barren of males. Females in these places are laying eggs and are quite abundant. On the other hand, a freshly cut field with adults eclosing has a high frequency of males. The frequency of females which have wet wings and are in copulation is very high. Yarmouth and Westley were examples of the latter. The other fields except Tracy were all examples of the former. Tracy was rather intermediate.

At Westley, in view of the correlation between highest frequency of white females and greatest fresh eclosions, a sample of wet-winged (copulated) females was obtained. The following results, which did not seem reasonable at the time, were obtained:

Westley, August 16, 1942		*N*
Free-flying	73.92 ± 1.33 percent	1,112
Wet-winged (copulated)	60.84 ± 3.31 percent	166

It is clear that the frequency is very different. The frequency of white eclosions is lower than the frequency of the free-flying females. This is true despite the contamination of the free-flying population with individuals also eclosed on that day but already in flight. The tentative conclusions were drawn either that white females did not mate so freely with the orange ones (negative sexual selection) or that the brood had already mostly eclosed, with the whites eclosing first. It was for the purpose of testing these conclusions that the San Fernando Valley population already mentioned was analyzed in September–October, 1942, when it was observed that the conditions were fortunately excellent at that one place. Earlier, attempts had been made to get frequencies of copulated females to compare with the general population frequency, this being an analysis of male sexual selection for the different colored females. Such attempts ended in failure, because the females don't often mate after the original copulation at eclosion. A sample was obtained in a field of old females near Rosedale, June 25, 1942, with the following results:

		N
Free-flying	53.33 ± 1.31 percent	1,440
Copulated but not newly emerged	54.17 ± 7.18 percent	48

The number is small but nearly the same in both cases. It is doubtful if the colored forms have any different sexual antipathy.

Two days after the sample described above was taken at Westley another sample was obtained. This time copulated females were more difficult to find. The suggestion is that the emergence was nearing its close. The results were:

Westley, August 18, 1942		*N*
Free-flying	71.12±1.30 percent	1,056
Copulated (with wet wings)	65.38±9.35 percent	26

The number of newly eclosed females is too low this time to have much significance. It is still lower than the general frequency. The frequency in the free-flying individuals has dropped 3 percent, but the significance is doubtful (not quite twice the standard error of the difference: 2.80±1.64 percent).

If the white females had eclosed in a higher frequency earlier in the general population emergence, it would be expected that the white frequency in the general population would drop daily just as it did in the San Fernando Valley population. The above 3 percent drop may represent this difference.

Toward the end of the adult emergence it would be expected that the frequency of white females in the newly eclosing individuals would be lower than that in the general population. This, too, is true just as it was in the San Fernando Valley populations.

On the same grounds, it would be expected that the area where the adult emergence is taking place in greatest quantity would have the highest white frequency. This is true. The central area where the white female frequency is highest is also the area where the adults were emerging in the greatest quantity (especially at Yarmouth and Westley). From this central point of greatest abundance, they were probably flying in all directions. The high frequency at Vernalis where there was no emergence noticeable was due to the trap effect of the "isolated" alfalfa field where the sample was obtained. Being between Vernalis and Yarmouth, it would attract adults flying away from those centers.

These data, therefore, suggest that samples taken at different times of the brood emergence may not be typical of the general region.

The average frequency for the North San Joaquin Valley area is 68.50±0.49 percent (N=9,024). The percentage will be compared with others later.

The Central San Joaquin Valley Area

Four frequency samples were obtained at variously-spaced locations between the North and the South San Joaquin Valley areas. The valley is flat the entire distance, but regions where alfalfa is grown are spotty. Therefore, some of the distance may be said to be a barrier to Colias movement by reason of space alone. The map (fig. 1) illustrates the locations of the places. The two southernmost of these localities have the lowest frequency but are not greatly different than would be expected, nor from what has been found in the more detailed zones. The 65.37±11.29 percent (N=891) Allensworth and the 64.42±8.50

percent (N=38) at Corcoran are close to the 64 percent average for the north end of the South San Joaquin Valley area. The Hanford 59.48±1.88 percent (N=686) and the Madera 61.72±1.69 percent (N=802) are the lowest north of Bakersfield. The following explanation may be suggested for these figures:

(a) These may be just chance changes in the gene frequencies at various places owing to lack of climatic differences between the localities.

(b) Alfalfa fields are not so common in this area, and the populations are more isolated. Therefore, the population size (abundance) may be smaller, allowing chance fluctuations greater leeway.

(c) The smaller population size may be sufficient to eliminate part of the selective advantage the white allele may have.

The Central San Joaquin Valley area as sampled by these four locations has a white female frequency lower than the north end of the South San Joaquin Valley, 62.52 percent versus 64.50 percent, which is just over twice the standard error of the difference. However, considering the South San Joaquin Valley as a whole, it is higher:

		N
North San Joaquin Valley	68.50±0.48 percent	9,324
Central San Joaquin Valley	62.52±0.97 percent	2,420
South San Joaquin Valley	59.39±0.41 percent	14,375
Total San Joaquin Valley	62.94±0.29 percent	26,119

A rise in white female frequency is apparent from south to the north in the valley.

The San Francisco Bay Area

Alfalfa is apparently grown commonly only at the southeastern portion of the Bay Valley. Hence, the sample was obtained there (fig. 1). Actually, several small areas were investigated, but only the total value is of reasonable size. The average frequency here of 66.04±3.70 percent (N=159) is in agreement with the 68.50±0.49 percent (N=9,324) average for the San Joaquin Valley at the same latitude.

A summary of the frequencies in four of the five provinces in California is given in table 6. The fifth province, the Great Basin, is not comparable and will be discussed below.

THE GREAT BASIN AREA

The Great Basin Area was sampled in two locations, Mono Lake Valley, Mono County, and Round Valley, Inyo County (map, fig. 1).

The Mono Lake area

Mono Lake is situated about 180 miles airline directly east of San Francisco (fig. 1). It is a Basin lake at an elevation of 6,300 feet above sea level and has no outlet. The country around it is very high. The Sierra Nevada Mountain range is a continuous wall on the west with peaks up to 13,000 feet in elevation, with no passes lower than 10,000 feet. Mountains 8,000 to 10,000 feet

TABLE 6

Summary of the white female frequencies in the orange race Colias chrysotheme populations in California west of the Sierra Nevada but including the southwestern desert areas.

LOCALITY	% WHITE FEMALES σ	N
1. San Francisco Bay	66.04±3.70	159
2. San Joaquin Valley		
North	68.50±0.48	9,324
Central	62.52±0.97	2,420
South	59.39±0.41	14,375
3. Coastal Southern California	29.86±0.34	18,617
4. Coachella-Imperial-Colorado River Valley area	16.31±0.82	2,035
Grand total	47.77±0.23	47,010

high surround the basin on the other three sides. The country is very arid due to the rain-shadow effect of the western mountain range. Winters are exceedingly cold, going well below 0°F, and snow falls for several months of the year if there is any precipitation at all. Summers are often exceedingly hot but sometimes it may be cold throughout the year. The air is always dry.

Meadows watered by springs and melting snow in the mountains occur in several places around the western edge of the lake. In these meadows, native clovers of various sorts grow, as well as some alfalfa mixed with red and white clover planted by the native residents. The agriculture of the region is primitive; the fields or meadows are grazed by sheep or cattle rather than being cut for hay as in the western valleys. In the meadows, one finds the yellow race Colias at certain times of the year and nearly everywhere one finds the orange race. In the wet meadows both the yellow and the orange race seem to concentrate in the largest numbers, and it is in two of these meadows that the frequencies of the white, yellow, and orange forms were obtained. The yellow form presents an entirely different problem from the white form and will be dealt with separately (HOVANITZ 1943c).[1]

The seasonal fluctuations in the white female frequency were great. Early in the season for that place the frequency was high; in the middle of the sum-

[1] The white females of the orange and yellow races in the Great Basin region are indistinguishable by phenotypical characters (HOVANITZ 1943b, 1943c). Therefore, without breeding, it is not definitely known whether they are of the one race or the other. The frequencies given in this paper are calculated as if they were all of the orange race. The reasons why it is not believed that extensive error is being introduced into the data are as follows: Breeding and museum data have suggested that the white form is nearly absent in the yellow race in the Mono Lake and Round Valley region. The presence of yellow race adults at Mono Lake, at least, is seasonally controlled (HOVANITZ 1943c). The presence or absence of yellow race adults at this locality has no influence on the fluctuation of the white female frequency. Since more than half of the adults at Round Valley are of the yellow race, the frequency here may be considerably in error. However, no white females from this place which have been bred have given entirely yellow progeny. Also, no yellow females used for breeding purposes from either of the two localities have produced a white form.

mer it was low. Again in the fall, the frequency rose again to nearly its early height. This change is seasonal frequency is illustrated on the graph (fig. 6) for the three years—1940, 1941 and 1942. The fundamental change is the same in all three years. The major portions of the samples making up the curves are statistically significant (table 7). At times it was very difficult to get a reasonable sample, and at first (until the middle of 1941) all samples were obtained by capturing the females with a net.

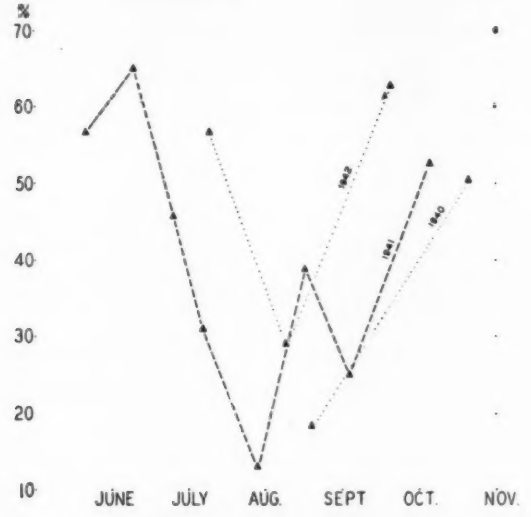

FIG. 6.—The frequencies of white females during the season at Mono Lake, California. The great seasonal change in frequency should be compared with that shown in figure 2.

By way of summary, the 1941 season started out in May and early June at 55–65 percent white females, then dropped through 45 percent in late June, 30 percent the first of July to a low of 15 percent at the end of July. This rose gradually through August and September to a high again of 52 percent in early October.

In 1942, the season started late at Mono Lake for the orange race. In early July, the frequency was at about 58 percent white. By the first of August this had dropped to at least 29 percent and rose again in late September to a high of 62 percent. The samples were not made at as frequent intervals during 1942 as in 1941; hence, the actual low point might have been missed. The 1940 collections are small but, nevertheless, follow the trend perfectly.

This great seasonal change in white female frequency is not duplicated in any of the populations considered heretofore (fig. 2). It is not an effect of the direct influence of the environment upon the phenotype of the adult, for the character is completely under genetic control (HOVANITZ 1943a).

A gene frequency alteration of the proportions needed to change the percentage of white females from 65 percent to 13 percent at Mono Lake is the same as would be necessary to change the population in the North San Joaquin Valley to equal those in the Imperial Valley. This change takes place in as short a period of time as one and one-half months. For the Mono Lake area, it takes place in time equal to the developing period for one generation.

TABLE 7

*The frequency of white females throughout the season at Mono Lake, Calif. This is the frequency as compared with the normal orange females of the orange race.**

DATE	% WHITE FEMALES σ	N
1940		
Aug. 11	18.2 ±11.1	11
Oct. 20	50.0 ±8.48	34
1941		
May 19	56.82±7.45	44
June 8	65.96±4.36	117
June 24	45.75±4.04	153
July 5	31.08±2.22	415
July 26	13.13±3.41	99
Aug. 15	38.74±4.56	111
Sept. 2	25.0	4
Oct. 4	52.27±7.45	44
1942		
July 7	56.30±3.21	238
Aug. 7	29.23±2.82	260
Sept. 16	62.89±3.46	194

* See footnote page 53.

Several possibilities to account for this rapid gene frequency change have been considered.

(a) That there is a tremendous environmental selection in the area against the recessive orange allele in winter due to the very cold winters and in favor of the recessive allele in summer due to the hot, dry weather. The selection during the winter is easy enough to imagine, but the summer change is so rapid that it does not seem possible.[2] Even with the aid of a possible homozygous lethal dominant, which would change the frequency from 66.7 percent to 50 percent in one generation, it is too great a change to expect. The climatic change is not that abrupt or extreme.

(b) That the homozygous dominant white female (and male) is lethal in warmer weather. This would reduce the population very rapidly from 66.7

[2] DOBZHANSKY (1943) has just shown some similar great changes in the genetic structure of wild populations. As in the present case, his data seem to be best explained by seasonal population migration rather than by seasonal selection.

percent to 50 percent to 28.6 percent to 25 percent, etc., per generation or, in terms of gene frequencies, from 33.3 percent to 25 percent to 14.3 percent, etc. (table 1). It would take more than nine generations to get from 65 percent to 13 percent at this rate, and only one generation is possible.

(c) That white females are at a predatory disadvantage and are likely to be eliminated in the summer more than at the colder parts of the year. The degree of bird attacks on adult butterflies of Colias was closely and carefully observed during the sampling of all the populations in 1941 and 1942. It must be considered that predatory attacks are negligible, though not completely absent.

(d) That there is an alternation of generations during the season such that spring and autumn individuals represent a really different population from the midsummer one. In some of its aspects, this is the conclusion accepted. However, it is not believed that both "populations" live and breed at Mono Lake. If they did there would have to be almost no intermixing in order to prevent gene interchange. No break of this sort is observed at Mono Lake in the orange race. Instead there is a population "high" in midsummer and decrease toward spring and fall, rather than discrete broods.

(e) That the individuals at Mono Lake have migrated there from some other place so that at certain times of the year the individuals are representative of some other locality. This would really be an alternation of "generations," at the locality. At first, this possibility seemed unlikely but later evidence has made it seem very credible. The reasons are as follows:

1. The exceptionally rapid gene-frequency alteration is most reasonable on these grounds. The presence of individuals from a high-frequency population at one time of the year and from a low-frequency population at another time is the easiest explanation.

2. Populations of high frequency (60 to 70 percent) exist just west of the Sierra Nevada from where migrations could have had their origin (San Joaquin Valley). The Great Basin and Mountain Region have a low dominant gene frequency (14 percent—N = 635) as judged by museum data (HOVANITZ 1943b). Nevada and eastern California have frequencies of 19 and 17 percent, respectively (N = 43 and 65). Much of the latter material is probably yellow race, since there is only 29 percent orange race calculated in the above figures. Apparently the environmental conditions in the dry mountain and basin region are most favorable for the lower dominant allele frequency. It is not believed that there is any migration of individuals into the Mono Lake area from low-frequency areas but rather that the low-frequency individuals represent the resident population.

3. When the white frequency is high, the individuals are invariably old and worn. When low, the individuals are much fresher and newly eclosed as if they had been bred in the vicinity.

4. At the times of high frequency early and late in the season the adults flying are of a phenotype development which could not have occurred in the cold weather of the Mono Lake climate. Were the pupae or larvae exposed to the cold night temperatures of the vicnity, they would be reduced forms com-

parable to "spring forms" in the low lands. A very few "spring form" individuals do occur early in the season and especially in the autumn. These are the resident individuals. Most adults at those times are worn "summer form" and were probably grown at a lower elevation. Later, as the resident population increases in size, it masks the high white frequency of the immigrant population. This would account for the gradual rise in the recessive orange allele frequency with a lack of a definite series of broods during the season as is found in the San Joaquin Valley (MICHELBACHER and SMITH unpublished). It was originally assumed that the adults found at Mono Lake in May were overwintering individuals because of the "summer" phenotype development they possessed. Such a supposition had been made as long ago as the nineteenth century by MEAD and EDWARDS (Edwards 1863–1898). They found that adults high in the Colorado Rockies early in the season were also "summer" individuals. The high improbability that Colias adults can overwinter in the true sense is shown by the length of time they can be kept in a cold room at extremely high humidities and normal low humidities. At 2°C, females will live inactive for three to four weeks or possibly a little longer at an extremely high humidity. At a normal low humidity they will die in one to two weeks at that temperature. The dryness of the Mono Lake atmosphere would kill the butterflies there quickly if the cold did not.

That Colias of the orange race can fly distances as great as the 100 miles from the San Joaquin Valley to Mono Lake is illustrated by the many migratory individuals taken in the far northern parts of North America (Hudson Bay, Northern Manitoba, Alberta, etc.), where overwintering populations do not exist. "Summer-form" individuals of the orange race have been taken in the New England states very early in the season. They must have flown in from the South. The ability to travel is great in the orange race of *Colias chrysotheme* (HOVANITZ 1943d). Summer form females have been observed laying eggs high in the San Jacinto mountains of Southern California in May and June where temperatures in the day are cool and at night near freezing. They could not have overwintered there but must have come from the alfalfa fields in the valleys below.

The orange race is fairly abundant in the Hudsonian and Alpine life-zones of the Sierra Nevada in midsummer. It is exceedingly doubtful that they could have overwintered in such a cold place but it is more likely that they have been re-introduced into the mountains seasonally. Apparently, a re-introduction takes place in the north such as in Alberta every few years (BOWMAN 1942). *Colias behri* is the resident Colias of the Hudsonian meadows in the Sierra Nevada, to which place it is restricted.

The question of why the Mono Lake population never reaches equilibrium with the San Joaquin populations probably involves several factors. The resident individuals may be better able to withstand the cold winters just as the orange race in the north-eastern United States can now better withstand the winters there. Or, the low frequency of the populations throughout the Basin and Mountain area may just absorb the excess white genes coming from the valley. Climatic selection in the area may be sufficient to reduce the frequency

after a period of time. Or, the low white allele frequency in the yellow race of the area may absorb and eliminate many of the genes through intercrossing. The yellow race is apparently better adapted to resident life in this region judging from its frequency of 71 percent for the mountain area in the museum data. Or, the dominant gene may have a disadvantage in the genome typical of the Basin region which would render the homozygote-dominant lethal or reduce the white heterozygote frequency.

The average frequency of white females at Mono Lake is 43.16 ± 1.18 percent ($N = 1,724$).

The Round Valley Area

Round Valley is south of Mono Lake 60 miles. It is part of Owens Valley and is 2,000 feet lower than Mono Lake, or 4,500 feet. The winters are not quite so cold a Mono Lake, and the summers are very much hotter. The warm season is several months longer. The general relationship to the Sierra Nevada range and San Joaquin Valley is the same as Mono Lake. There are, however, more alfalfa fields in the vicinity, especially to the south in Owens Valley. Round Valley itself is a big meadow with mixed native clovers, white clover, red clover, alfalfa, and grass fields kept green by irrigation and springs.

TABLE 8

*The frequency of white females throughout the season at Round Valley, Calif. This is the frequency as compared with the normal orange females of the orange race. However, in 1942 66 per cent of the females other than white at this place were of the yellow race.**

DATE	% WHITE FEMALES σ	N
1941, season from May–Oct.	35.13 ± 7.84	37
1942		
April 1	28.6	7
April 25	50.0 ± 10.2	24
June 12	53.85 ± 9.78	26
July 8	58.46 ± 4.32	130
Aug. 6	65.22	8
Sept. 16	51.61 ± 8.98	31
Total	56.64 ± 3.42	226
1941–42 total	53.61 ± 3.04	263

* See footnote page 53.

The frequencies of white females obtained at this locality were purely incidental to the study of the orange-yellow relationships. Therefore, the numbers in the samples are small (table 8). Apparently, however, the frequency may not fluctuate seasonally as at Mono Lake, since in July of 1942 a frequency of 58 percent was procured. This may be accounted for by the more desert environment around Round Valley such that the orange race population is completely restricted to alfalfa fields. Apparently, there is a great migration of Colias over the Sierra Nevada to Round Valley just as to Mono Lake. With-

out a native population of orange race other than that one in alfalfa fields, the high white frequency is not reduced much by swamping of the genes.

The average of white females during 1941 and 1942 was 53.61 ±3.04 percent (N = 263). This is statistically higher than Mono Lake's 43.16 ± 1.18 percent (N = 1,724). The north-south rule is reversed, and the climatic expectancies are also reversed. However, possible reasons for this condition have already been covered. (Probable lack of resident population.) The frequency of the total for Mono Lake and Round Valley is 44.53 ± 1.06 percent (N = 1,987). This total is much higher than the 17 percent determined from museum material for the eastern Sierra Nevada, but the latter was based on 70 percent yellow race contamination. White females in the yellow race at Mono Lake and Round Valley are either totally absent or close to it.

SUMMARY AND CONCLUSIONS

The frequency distribution of white females in the orange race of *Colias chrysotheme* (= *eurytheme*) has been shown to change from 74 percent in the North San Joaquin Valley to 13 percent in the Imperial Valley. This change in frequency has been shown to be largely correlated with the change in climatic conditions through the area.

The greatest change in frequency between populations takes place where there is complete isolation between populations as well as a climatic difference. However, an environmental selection for the alleles controlling the characters is shown to be present even when no isolation other than distance exists between populations (San Joaquin Valley).

Wild population changes in gene frequency of various magnitudes have been illustrated. Most of these are shown to be due to population movement and others to a differential development rate of the genotypes.

The causes of population differences have been shown to lie in any of the following: (a) possible random deviation, (b) climatic selection, (c) differential population movement and migration, and (d) different age of the adult population after eclosion.

The faster development rate of white females (heterozygous plus homozygous dominant) over the orange (homozygous recessive) is deduced from some of the population analyses.

No differential sexual selection by the males for the polymorphic females is noted.

The calculation of actual gene frequencies from wild population phenotype frequencies in any organism can never be accurate so long as knowledge of the viability of the three genotypes ($p^2+2pg+q^2$) under known environmental conditions cannot be controlled; only good estimates can be made of the true gene frequency.

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X-RAY AND ULTRAVIOLET STUDIES ON POLLEN TUBE CHROMOSOMES. I. THE EFFECT OF ULTRAVIOLET (2537 Å) ON X-RAY-INDUCED CHROMOSOMAL ABERRATIONS[1]

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INTRODUCTION

THE direct approach to the problem of the mechanism of chromosome breakage and reattachment—namely, an analysis of the end results following radiation—has not as yet yielded a wholly satisfying answer, although our understanding of the limitations within which the mechanism operates has been considerably broadened, and its complexity has been recognized. An indirect approach has been made by determining the effect on breakage of varying the environmental conditions before, during, and after radiation. MICKEY (1938), SAX and ENZMANN (1939), and FABERGÉ (1940) have demonstrated that high temperatures reduce the total observed X-ray breakage, although MICKEY'S results seem doubtful in view of the more recent data of MULLER (1940). The plant data are explained by SAX and ENZMANN as resulting from an increased rate of restitution, thus leaving fewer broken ends available for reattachment. More recently SAX (1943) has shown that centrifuging during radiation increases the number of breaks; this, he feels, is due to the increased stresses placed on the chromosome during the period of breakage and reattachment.

With the recognition that a series of canalized events must bridge the gap between the initiating ionization, which is on an atomic level, and the realization of the complete break and its later reattachment (or restitution), which by comparison are on a macroscopic level (DELBRÜCK 1940), the indirect method of approach to this problem assumes considerable importance. By breaking down the series of events into separate units (at present only a theoretical possibility), a more thorough study could be made of the nature of the disturbances produced by ionization as well as of the conditions encouraging or discouraging breakage, restitution, and reattachment of broken ends. KAUFMANN (1941), recognizing this, has instituted investigations with infrared designed to alter if possible the irradiated sperm of Drosophila during the time between radiation and fertilization. The results of KAUFMANN'S study, however, have not as yet been published.

A series of preliminary experiments have been carried out to determine the effect of short-wave ultraviolet (2537 Å) on X-rayed pollen tube chromosomes of Tradescantia. It will be recognized that the more precise the tool used in such a study, the more informative will be the data obtained. Ultraviolet, in this respect, possesses several unique advantages. In addition to the fact that it can be successfully combined with X-rays by employing the pollen tube technique (SWANSON 1940a), any effect that it might have on X-ray breakage

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or reattachment would extend the possibility of securing an additional spectral curve (cf. STADLER 1939), leading perhaps to the identification of chromosomal constituents intimately concerned with such phenomena. Ultraviolet also yields data which may be subjected to strict quantitative analysis.

The data presented in this paper indicate that short-wave ultraviolet, applied within certain temporal limits relative to X-raying, can lead to a considerable inhibition of X-ray induced breaks, the degree of inhibition being dependent upon the ultraviolet dosage and the time of application. The fact that ultraviolet, like X-rays, produces chromosomal aberrations introduces certain complications, but since the changes induced by ultraviolet are for the most part of a different nature from those induced by X-rays, the distinction between them is fairly readily made and may in turn be used to advantage in further distinguishing between the effect of these two agents.

MATERIALS AND METHODS

A clonal line of *Tradescantia paludosa* Anders. & Woodson was used for this study. The data were derived from the irradiation of pollen tube chromosomes, using a technique previously described (SWANSON 1940a). The X-ray dosage was given at a rate of 123.6 r/min. from a Coolidge tube operating at 60 kv. The pollen tubes were held at 10 inches from the target. The ultraviolet source was a low pressure arc (Hanovia SC-2537), operating at 7500 volts and 120 milliamperes and emitting the greater part of its radiation at the 2537 mercury line. All radiations were carried out with the slides on which the pollen tubes were growing in a moist chamber fitted with an inch-deep water cell of fused quartz as a window. This permitted prolonged exposures without danger of desiccating the pollen tubes. Unfortunately no device was available for intensity measurements of the ultraviolet so that the dose can be given only in seconds. The data were derived from two experiments carried out during the summer and fall of 1942, each involving approximately the same number of chromosomes. Each figure in the total chromosome columns (tables 1 and 2) represents, on an average, an analysis of five slides. Since the results of the two experiments were quite similar in most respects, the data were lumped.

It should be emphasized here that ultraviolet-induced breaks can involve but a single chromatid at any one locus, with the expression of the break ranging from a partial lesion of the chromatid to complete detachment of the fragment. For the most part, the fragments seem to be separated from the remainder of the chromosome by an achromatic lesion (see fig. B, C, D, E, SWANSON 1942). The technique employed does not permit a further study of the deletions, but the similarity of previous ultraviolet studies (SWANSON 1940b, 1942) with the cytogenetic results of Stadler and his co-workers (STADLER 1939, 1941) leaves little doubt but that they are true deletions. To insure uniformity in scoring, only those breaks showing a clear lesion extending through the entire diameter of the chromatid were classified as single deletions. This method was also followed in scoring X-ray breaks of a similar nature. Where the chromosomes were exposed to both types of radiation it was not possible to distinguish by vision between the X-ray-induced and the ultraviolet-induced single deletions.

OBSERVATIONS

The effect of ultraviolet pre-treatment

The effects of ultraviolet on the production of X-ray breaks may be determined by irradiations before, during, or after X-ray treatments. Since technical difficulties precluded an ultraviolet treatment during X-radiation, only pre- and post-treatments were attempted.

TABLE 1

Effect of ultraviolet pre-treatment on the production of x-ray breaks (%).

TREATMENT*	SINGLE DELETIONS		DOUBLE DELETIONS		TRANSLOCATIONS		TOTAL BREAKS		TOTAL CHR.
	OBSERVED	EXPECTED	OBSERVED	EXPECTED	OBSERVED	EXPECTED	OBSERVED	EXPECTED	
uv/7½″/1 hr.	.39	—	—	—	—	—	.39	—	3084
uv/15″/1 hr.	1.01	—	.08	—	—	—	1.09	—	2376
uv/30″/1 hr.	2.25	—	—	—	—	—	2.25	—	2034
uv/60″/1 hr.	3.05	—	—	—	—	—	3.05	—	3540
x/2′/2 hr.	3.51	—	1.05	—	1.89	—	6.45	—	5304
x/3′/2 hr.	4.70	—	1.94	—	3.71	—	10.35	—	2532
uv/7½″/1 hr.+x/2′/2 hr.	2.53	3.90	.80	1.05**	2.53	1.89	5.86	6.84	2526
uv/15″/1 hr.+x/2′/2 hr.	2.59	4.52	1.11	1.05	.74	1.89	4.44	7.54	2430
uv/30″/1 hr.+x/2′/2 hr.	1.73	5.76	.78	1.05	.17	1.89	2.68	8.70	2310
uv/60″/1 hr.+x/2′/2 hr.	3.01	6.56	.35	1.05	.35	1.89	3.72	9.50	2256
uv/30″/1 hr.+x/3′/2 hr.	2.50	6.95	.81	1.94	.94	3.71	4.25	12.60	2964
uv/60″/1 hr.+x/3′/2 hr.	3.13	7.75	.62	1.94	.00	3.71	3.75	13.40	1920

* The symbols under the "Treatment" column are to be interpreted as follows: for example, uv/7½″/1 hr.+x/2′/2 hr. means a 7½ sec. dose of ultraviolet at 1 hour after the pollen was sown followed by a 2 min. X-ray treatment at 2 hours after the pollen was sown.

** The double deletions observed in the uv data have not been calculated into the "expected" values.

Table 1 presents the data derived from a series of pre-treatments in which the ultraviolet dose was varied and the time of treatment was kept constant. The ultraviolet was given one hour after the pollen tubes had been placed on the agar medium and one hour before X-raying. As judged by the percentages of the total number of chromosome breaks, the data show that such a pre-treatment exerts a decided inhibitory effect on the realization of X-ray breaks, the observed percentage being clearly lower than that expected were the breaks cumulative. Since there is no apparent reason at this time for assuming that the X-rays exert an influence on the production of ultraviolet-induced breaks, it is quite possible that the reduction, or inhibitory effect, is entirely at the expense of the X-ray induced types.

The inhibitory effect is increased with an increase in the ultraviolet dosage. It cannot as yet be stated whether the amount of inhibition is proportional to the dosage of ultraviolet applied. Indeed there appears to be a saturation effect operative at the higher ultraviolet (30 and 60 sec.) doses, as indicated by a leveling off in the amount of reduction. This is further suggested by the results obtained when the X-ray dosage is increased to a 3-min. (370.8 r) exposure. With a 60-sec. ultraviolet pre-treatment, there is no appreciable increase in total breaks (3.75 percent) over that found at the 2-min. X-ray dose (3.72 percent).

By breaking down the data for the individual types of breaks into percentage figures, it is possible to gauge more exactly the degree to which each is affected by the ultraviolet pre-treatment. Both observed and expected percentages are given. The data show that the various types—single deletions, double deletions, and translocations—do not behave in like manner, suggesting a differential reactivity. The single deletions show a reduction at the lowest ultraviolet dose employed, and they become gradually reduced in number until at the 30- and 60-sec. doses, they are to be found in no greater frequency than that expected from the ultraviolet alone. The increase in single deletions at the 60-sec. dose can be largely attributed to the increase in ultraviolet dosage. The data seem to suggest, therefore, that the X-ray induced single deletions have been completely eliminated, but the inability to distinguish between ultraviolet-induced and X-ray-induced deletions leaves this somewhat in doubt.

The inhibition of translocations by the pre-treatment is more pronounced than that of the double deletions. From the data there appears to be a rise in translocations following the $7\frac{1}{2}$-sec. ultraviolet dose, but since this is largely due to an increase found in only one of the two experiments, it is of doubtful significance. A drop in percentage is noticeable as the dose is increased, with an apparent leveling off at the higher doses as the result, probably, of a saturation effect. Raising the X-ray dose to a 3-min. exposure did not materially increase the production of translocations when the ultraviolet (at the 30- and 60-sec. doses) was held constant. A 60-sec. dose followed by a 3-min. X-ray exposure resulted in no detectable translocations.

Double deletions are the least affected of the various aberrations. Not until the ultraviolet was increased to 30- and 60-sec. doses (with the 2-min. X-ray exposure) was an appreciable effect noticed. On the other hand, the lowered percentage found following a $7\frac{1}{2}$-sec. dose indicates that some effect may be produced even by small amounts of ultraviolet. At the 60-sec. doses, one-third of the expected double deletions are still produced, and no saturation effect was evident. An additional increment of ultraviolet would probably be necessary to completely eliminate the double deletions. It is significant to note that at the 60-sec. dose, the double deletions, for the most part, were of a different type than that usually induced by X-rays. The sister chromatids appeared to be broken at identical loci, but instead of uniting to form the customary U-shaped fragment and dicentric chromatid, the broken ends were held in place without union and remained simply as two single deletions. It appears, therefore, that the ultraviolet not only reduces the number of double deletions, but prevents those that are produced from uniting broken ends as generally occurs following X-radiation.

The effect of ultraviolet post-treatment

To test the effect of ultraviolet when applied after the X-ray treatment had been given, post-treatments were made immediately after X-raying, and then at one-half and one hour intervals. For the sake of comparison, the results of a pre-treatment given immediately prior to X-raying are included (table 2).

The ultraviolet dose was kept constant at 30-sec., but since the ultraviolet breakage rate varies with the time of prophase development (SWANSON 1942), it was necessary to determine the effect of the ultraviolet at the various time intervals used in the post-treatments. All the X-ray exposures in this experiment were of 2-min. duration.

TABLE 2

Effect of ultraviolet post-treatment on the production of X-ray breaks (%).

TREATMENT	SINGLE DELETIONS		DOUBLE DELETIONS		TRANSLOCATIONS		TOTAL BREAKS		TOTAL CHR.
	OBSERVED	EXPECTED	OBSERVED	EXPECTED	OBSERVED	EXPECTED	OBSERVED	EXPECTED	
uv/30″/2 hr.	3.17	—	—	—	—	—	3.17	—	1704
uv/30″/2½ hr.	2.99	—	—	—	—	—	2.99	—	2004
uv/30″/3 hr.	2.79	—	—	—	—	—	2.79	—	2436
x/2′/2 hr.	3.51	—	1.05	—	1.89	—	6.45	—	5304
uv/30″/2 hr.+x/2′/2 hr.	2.25	6.66	1.46	1.05	.45	1.89	4.17	9.62	1776
x/2′/2 hr.+uv/30″/2 hr.	3.29	6.66	1.17	1.05	.00	1.89	4.46	9.62	2556
x/2′/2 hr.+uv/30″/2½ hr.	2.25	6.49	1.02	1.05	1.43	1.89	4.70	9.44	1956
x/2′/2 hr.+uv/30″/3 hr.	3.72	6.29	1.68	1.05	2.32	1.89	7.72	9.24	1922

The data in the total break column indicate that short-wave ultraviolet can materially inhibit the degree of X-ray breakage even when applied as a post-treatment. This indicates that not all of the X-ray breaks and rearrangements are immediately realized, a conclusion that has been previously drawn by other investigators using other methods (MULLER 1940; KAUFMANN 1941; SAX 1930). Double deletions appear to be little affected by a post-treatment of ultraviolet. If anything, there is a slight increase, although the significance of this needs further verification. Translocations are markedly reduced when the ultraviolet either just precedes or follows the X-raying, but if a one-half hour interval is allowed between the two treatments, the reduction is slight. These data are in keeping with the conclusion drawn by SAX (1940) that most reattachments in the microspores of Tradescantia are completed within an hour's time.

Single deletions, on the other hand, show a decrease throughout the entire range of post-treatments. The inhibition appeared to be less marked after one hour had intervened between the X-raying and the ultraviolet treatment. The explanation for this continued reduction is not readily apparent, but it may be that the ultraviolet leads to an increased rate of restitution even if applied long after the X-raying.

The lack of effect of the ultraviolet on double deletions seems to indicate that this type of breakage is realized at the time of radiation, having no appreciable delay between breakage and reattachment. The data in table 2 also suggest that the inhibitory action of ultraviolet is not immediately effective. Pre-treatments given an hour before X-radiation are effective in reducing the double deletions, but if given just prior to the X-rays, no reduction is evidenced. It is possible that certain gelation phenomena may be involved in this reaction, with completion of the reaction requiring a certain time interval.

DISCUSSION

The data presented show that, within limits, pre- or post-treatment with short-wave ultraviolet leads to a reduction in the number of chromosome breaks realized from a given X-ray dose. The types of breaks react variously to these treatments, but by varying the time of treatment and the dosage, all can be materially inhibited. The change produced in the chromosome or the cytoplasm by the ultraviolet treatment is at present obscure, but it appears most likely that some constituent of the chromosome undergoes an alteration on absorption of the ultraviolet. Previously published data (SWANSON 1942) suggest that this constituent might be the chromosome matrix. When subjected to short-wave radiation (2537 Å) of sufficient dosage, the matrix tends to become quite conspicuous, the chromosomes appear to be contracted more than usual, and they fail to separate readily into two distinct and free chromatids. With longer wave lengths (2967 and 3022 Å) this does not occur even though the dosage, in ergs, be increased 20-fold. (It should be pointed out, however, that in this study the ultraviolet doses were not sufficiently heavy to produce a visible change in the matrix.)

The differential inhibition of the single deletions and translocations on the one hand and the double deletions on the other lends some support to this matrical conception. The ease with which the single deletions and translocations are inhibited suggests that the ultraviolet prevents single breaks from entering into new associations by interfering with the normal behavior of the matrix, possibly by increased gelation of the matrical substance which makes it more resistant to breakage. It has been emphasized before that breaks produced by ultraviolet do not as a rule enter into new rearrangements, nor do the single deletions become dislocated from the remainder of the chromosome but remain attached and in position, separated from the rest of the chromosome only by an achromatic lesion. This behavior again in all probability relates to the matrix, for, as MULLER (1940) points out, the ultraviolet cannot disrupt the matrix to produce a "thoroughgoing break," whereas an X-ray break with its subsequent reattachment must involve both matrix and chromonema. Double deletions do not appear to be so affected. Despite the ultraviolet treatment, these breaks are regularly, although sometimes less frequently, produced, and they involve both matrix and chromonema. Only at the higher pre-treatment doses do the double deletions not show the characteristic U-shaped fragment and dicentric chromatid, but show instead parallel chromatids each having a deleted portion with the breaks at identical loci. The failure of the fragments to unite to form a U-shaped fragment very likely may be attributed to interference by an altered matrix. The fact that double deletions may be formed when single deletions and translocations are readily inhibited suggests that they might be formed first within the fused matrices of the two chromatids. The gap in the broken chromonemata will form a weakened locus which will lead to a disruption of the matrix. This is supported to a certain extent by observations which show that while U-shaped fragments are produced after, say, a 30-sec. ultraviolet pre-treatment, they are usually attached to the remainder of the chromosome by a transparent connection which is undoubtedly

derived from, or is a portion of, the matrix. Where X-ray breaks are prevented by the matrix from entering new associations with other similar breaks, they probably undergo restitution, thus reducing the number of expected breaks visible at metaphase.

In connection with the assumption of a matrical control of chromosome breakage, it is of interest to consider two groups of seemingly conflicting observations. Visual observations of anaphase chromosomes leave little doubt that they possess a double structure—that is, the chromonema is split into two distinct chromatids. Yet, on the other hand, X-ray data (SAX 1940, and others) show that the chromosome during the resting stage behaves as a single unit in breakage and reunion. One is therefore forced to conclude that the subdivision of the chromosome does not determine the type of breakage and reunion which will result from X-radiation. The only remaining chromosomal structure of which we are aware is the matrix. Can the behavior of the matrix, whose structure and function are as yet but poorly understood, adequately account for chromosome behavior during and after radiation? Obviously our present knowledge does not permit of generalizations, but if it is assumed that the matrix remains undivided until prophase (apparently it is in anaphase) and that during the resting stage it maintains the unity of the chromosome as a single whole, then X-radiation would produce only chromosome, or 1-thread, breaks up until the time of matrix division, after which only chromatid, or two-thread, breaks would be produced. This transition period occurs at about 30 hours before metaphase (SAX 1940) in Tradescantia microspores.

That the ultraviolet post-treatment leads to increased restitution is suggested by the data in table 2. The single deletions still show considerable reduction even when the post-treatment is given an hour after X-radiation. Since the double deletions seem to be quickly realized and the translocations are completed within an hour after X-raying, it appears reasonable to assume that the single deletions are also realized within this same time interval. That the observed percentage is much below that expected, however, indicates that restitution has taken place after the ultraviolet has been given. MCCLINTOCK (1942) has beautifully demonstrated in maize, as have MULLER (1940) and KAUFMANN (1941) in Drosophila, that broken ends, or "potential breaks," are capable of remaining "open" for a considerable length of time. Restitution must also be possible under the same conditions, and in the pollen tubes of Tradescantia the ultraviolet appears to hasten this type of union.

In connection with the observations made by STADLER (1939, 1941), MULLER (1940), and SWANSON (1940b, 1942) to the effect that the ultraviolet breaks are qualitatively different from those produced by X-rays, it is significant to note that the single deletions produced by these two agents are not cumulative, even though each agent by itself can induce this type of break. Were they produced by the same mechanism, or were the broken ends similar, the single deletions should be cumulative. Since they are not, there is good reason, therefore, for believing that, despite their similar appearance, they are produced by different mechanisms, and that the manner of production determines the stability of the broken ends (cf. MCCLINTOCK 1941).

SUMMARY

A study of the effect of short wave length ultraviolet (2537 Å) on the production of X-ray breaks in the pollen tube chromosomes of Tradescantia led to the following conclusions:

Pre-treatment one hour before X-raying leads to an inhibition of all types of visible X-ray breaks. Single deletions and translocations are more affected than are double deletions.

The degree of inhibition depends upon the dosage of ultraviolet used, although the exact proportionality is not known.

Post-treatment has no inhibitory effect on double deletions regardless of time of application. Translocations show considerable inhibition if the post-treatment is given immediately after X-radiation, slight inhibition if given one-half hour later, no inhibition if given one hour later. Single deletions are inhibited even after the one hour post-treatment, suggesting that the ultraviolet facilitates restitution.

It is suggested that the action of ultraviolet is not immediate, but that a certain time interval is required before it becomes effective. A further suggestion is made that the inhibition caused by the ultraviolet results from an effect produced on the chromosome matrix which leads to its greater resistance to X-ray breakage.

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INDUCED TETRAPLOIDS OF A SELF STERILE OENOTHERA

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INTRODUCTION

SELF sterility of a type similar to that reported by EAST and MANGELSDORF (1925) for Nicotiana has been found in *Oenothera organensis* by EMERSON (1938). In a survey of self sterility in the flowering plants, EAST (1940) states, "DR. STERLING EMERSON, who had made many observations, writes that he believes that it is safe to conclude that the species (of Oenothera) belonging to the sections Euoenothera (Onagra) and Raimannia are all self fertile." He says that EMERSON thinks *O. organensis* should be in a new section, separate from either Euoenothera or Raimannia. He also mentions data of EMERSON'S (apparently not yet published) indicating self sterility in *O.* (*Anogra*) *californica*, *O.* (*Pachylophus*) *caespitosa* var. *marginata* and *O.* (*Lavauxia*) *acaulis*. *O.* (*Megapterium*) *missouriensis* has been reported by GATES (1939) to be self sterile. Several species included by MUNZ (1931) in the subgenus Anogra have been grown by the author, and preliminary studies indicate that the following species are probably self sterile: *O. latifolia* (Rydb.) Munz, *O. pallida* Lindl., *O. runcinata* (Engelm.) Munz, and *O. trichocalyx* Nutt. All of these are perennial; one annual species, *O. deltoides* var. *cognata* (Jeps.) Munz, was also grown, and it proved very definitely self fertile.

Two races of the subgenus Raimannia—*O. heterophylla Kaicaster* and *O. rhombipetala Bridgeport*—may now be added to this list, since all attempts by the author to produce seed by selfing have met with complete failure, whereas with only a few exceptions all plants in each culture have been inter-fertile. The general cytogenetic characteristics of these races have been described in another paper by the author to be published later; it is there pointed out that another race of *rhombipetala* has proved to be self fertile, and as would be expected, hybrids of the self sterile and self fertile races are self fertile. *O. heterophylla* and *O. rhombipetala* together constitute a definite and distinct section of the Raimannias, especially characterized by the persistence of their flowers and the clustered condition of their buds in the inflorescences. Among the Raimannias, however, they are perhaps the least like the self sterile *O. organensis* in external appearance.

EXPERIMENTS AND DISCUSSION

One of these self sterile Raimannias, *O. rhombipetala Bridgeport* was treated with colchicine, and, as has been described in a previous paper (HECHT 1942), tetraploids were obtained. STOUT and CHANDLER (1941) have reported a change to self fertility on the part of a tetraploid Petunia which is self sterile as a diploid. In *O. rhombipetala Bridgeport*, on the contrary, the tetraploid plants obtained from the colchicine-treated seeds, and the next two generations raised from these, have proved to be completely self sterile. (LEWIS and MOD-

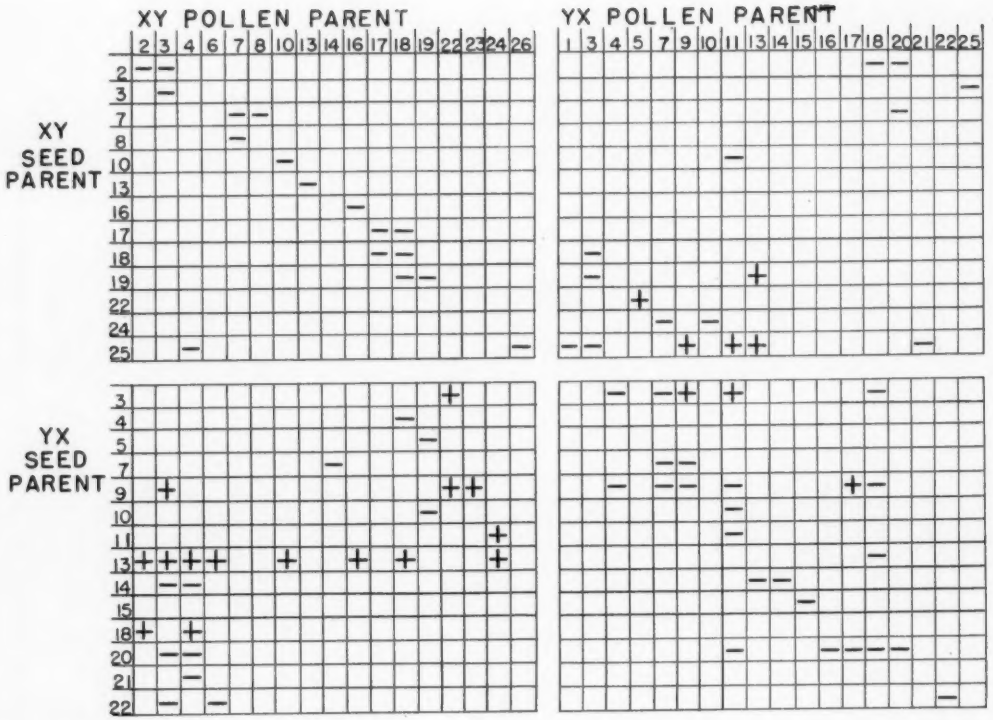
XY POLLEN PARENT
YX POLLEN PARENT
XY SEED PARENT
YX SEED PARENT

FIG. 1

LIBOWSKA (1943) have obtained tetraploids by colchicine treatment of *O. organensis*, and these tetraploids also are self sterile.) The genetic behavior of the first of these latter two generations will be discussed here; this generation consisted of the progeny of a single cross between two self sterile tetraploid individuals and its reciprocal, and preliminary studies indicate a self sterility mechanism similar to that proposed by EAST and MANGELSDORF (1925). Figure 1 presents the results of the crosses within this generation.

It will first be assumed that the two tetraploid plants (X and Y), which were obtained following colchicine treatment, and which were used as parents for the second generation, were obtained from diploid seeds (x and y) differing in only one self sterility allele, as follows: x, S_1S_2; y, S_1S_3. It will be shown later that this was probably the actual condition—that it is unlikely that y was S_3S_4. With respect to the self sterility alleles, the two first-generation tetraploids must have had the following formulae: X, $S_1S_1S_2S_2$; Y, $S_1S_1S_3S_3$. In the cross of X as seed parent and Y as pollen parent (X×Y), three types of eggs and three types of pollen would be expected, in the following ratios:

	EGGS		POLLEN
1	S_1 S_1	1	S_1 S_1
2	S_1 S_2	2	S_1 S_3
1	S_2 S_2	1	S_3 S_3

Since only those pollen grains which do not contain self sterility alleles that are present in the style of the seed parent will grow through the style (EAST and MANGELSDORF 1925), only the S_3S_3 grains will function, and three classes (A, B, and C) of progeny will be expected, in the following ratios:

1 (A)	S_1 S_1 S_3 S_3
2 (B)	S_1 S_2 S_3 S_3
1 (C)	S_2 S_2 S_3 S_3

On similar grounds the reciprocal cross (Y×X) might likewise be expected to include three classes of progeny, (A′, B′, and C′), as follows:

1 (A′)	S_1 S_1 S_2 S_2
2 (B′)	S_1 S_3 S_2 S_2
1 (C′)	S_3 S_3 S_2 S_2

The progeny of X×Y will be called XY plants, and those of Y×X, YX plants. Figure 2 shows that only two out of 16 random pollinations among XY plants (or YX plants), and only five out of 16 between XY and YX plants (crossed either way) might be expected to set seed.

Preliminary analyses show that these relationships do hold. Some plants (classes B and B′) never set seed, whereas others (classes A and A′) succeed for all crosses with pollen from plants of the reciprocal cross. Class C plants set seed only when crossed with pollen from an A or A′ class plant. In figure 1, XY plants 19 and 25 must be class C, since they give positive results with some YX plants and negative results with others. Class A plants should set seed with all YX plants, class B plants with none of them. It follows that YX

plants 9, 11, and 13 are class A′ plants, since class C will set seed only with class A′ plants in this cross. Plant 3 of YX must, therefore, be C′, since this is the only class which will set seed with class A′ pollen when YX plants are inbred. Then plant 22 of XY must be A, since its pollen produces seeds in a class C′ plant. Because pollen from plant 4 of YX gives no seed with plant 9 of YX (class A′), it must be class A′ or B′. It cannot be A′, however, since it fails to set seed when used as egg parent in a cross with an XY plant. It is, therefore, class B′. The same argument applies to plant 7 of YX, which is also class B′. Since plant 10 of XY fails to set seed with pollen of plant 11 of YX (class A′), it must be class B. Plant 20 of YX sets no seed with pollen of XY plants. It could be, therefore, a class B′ or C′ plant. If it were C′, however, it should set seed with YX plant 11 (class A′). Since it does not, it is class B′. Plant 17 of YX, when used as ♂, produces seed on class A′ plant 9, but none on class B′ plant 20. It must, therefore, belong to class C′. Plant 14 of YX fails to set

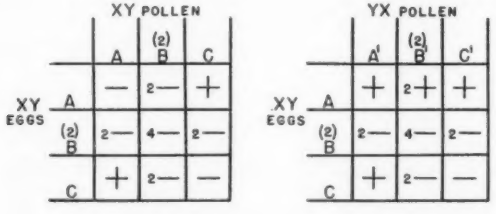

FIG. 2

seed with pollen from plant 13 of YX (class A′). It is, therefore, class A′ or B′. Since it sets no seed with YX pollen, however, it cannot be A′ and must be B′. Plant 10 of YX sets no seed with pollen of XY plant 19 (class C). It must, therefore, be class B′ or C′. If it were C′, however, it would set seed with plant 11 of YX (class A′)—but this does not happen. It is, therefore, class B′. Plant 18 of YX sets seed with pollen of plants 2 and 4 of XY (classes B′ or C′). It must, therefore, be class A′. Plant 2 of XY must be class B, for it fails to set seed with pollen from YX18 (A′) and YX20 (B′).

This completes the list of the plants in figure 1 whose types can be determined definitely on the basis of the data accumulated. Other plants can be placed in either of two classes and a few, because of lack of data, cannot be placed at all. Only one discrepancy appears in the figure; otherwise the scheme is entirely consistent. Plant 3 of YX (class C′), used as ♀, should have set seed when pollinated by plant 18 of YX (class A′). Attempts to make this cross were made during hot, dry weather, when the plants were in poor condition. It is altogether probable that failure to obtain seed in this case was a result of physiological conditions determined by the environment, and not the result of genetic factors. That this one discrepancy is probably of no significance is suggested by the fact that the scheme in other respects checks perfectly and also by the fact, shown below, that the results cannot be explained

by the assumption that the original plants differed by more than one sterility allele.

If additional time had been available, further crosses would have been made to test the hypothesis explained above, thus rendering the proof more convincing. The necessity of entering the armed forces has made this impossible for the time being, however, and it is thought that the evidence is sufficient to warrant placing the facts at the disposal of other workers.

If the original plants had differed not by one, but by both self sterility alleles, so that the two original tetraploids had the following formulae: X, $S_1S_1S_2S_2$; Y, $S_3S_3S_4S_4$; then nine classes of progeny would have been expected from the cross of X×Y (or Y×X), in the following ratio:

1	$S_1\ S_1\ S_3\ S_3$	2	$S_1\ S_1\ S_3\ S_4$	1	$S_1\ S_1\ S_4\ S_4$
2	$S_1\ S_2\ S_3\ S_3$	4	$S_1\ S_2\ S_3\ S_4$	2	$S_1\ S_2\ S_4\ S_4$
1	$S_2\ S_2\ S_3\ S_3$	2	$S_2\ S_2\ S_3\ S_4$	1	$S_2\ S_2\ S_4\ S_4$

It has been shown that class A (or A′) plants are characterized by being universal receivers of pollen from plants of the reciprocal cross. The latter scheme—with plant Y differing from plant X in two self sterility alleles—does not allow for class A plants. Since at least one class A plant did occur in our cultures, this latter scheme does not fit the facts, and it may, therefore, be concluded that the diploid x and y plants differed with respect to only one self sterility allele.

SUMMARY

Tetraploidy has been induced in a self sterile race of *Oenothera rhombipetala* by treatment with colchicine. An analysis of the inheritance of the self sterility alleles in the tetraploids revealed a self sterility mechanism based upon opposing sterility alleles according to the general scheme of EAST and MANGELSDORF. Successful matings resulted only when the potential seed parent contained none of the self sterility alleles present in the pollen. The situation is satisfactorily explained by assuming that the original diploid plants which became tetraploid following colchicine treatment differed from each other by only a single self sterility allele.

ACKNOWLEDGMENT

This study is a part of the general program of cytogenetic research on Oenothera which is being carried out at INDIANA UNIVERSITY under a grant from the ROCKEFELLER FOUNDATION to PROFESSOR RALPH E. CLELAND. The writer wishes to acknowledge his appreciation to PROFESSOR CLELAND for advice and assistance during the course of this investigation.

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CHROMOSOMES AND SEX DETERMINATION IN THE PARASITIC HYMENOPTERON TELENOMUS FARIAI (Lima)

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INTRODUCTION

THE problem of sex determination in Hymenoptera has attracted the attention of numerous investigators. Since in these insects, females are diploid and males are haploid, it has been thought for a long time that a single genome produces a male and two genomes a female. However, WHITING (1939, 1940a, 1943) and BOSTIAN (1939) have shown that in the parasitic wasp *Habrobracon juglandis* there exists a series of sex determining alleles xa, xb, xc, etc., any two of which produce a female (xaxb, xaxc, xbxc, etc.), while a haploid organism carrying any one allele is a male. This mechanism of sex determination works best in a population having a variety of alleles, since in such a population diploid individuals homozygous for any one allele (xaxa, xbxb, etc.) are seldom produced. Such homozygotes arise, however, in inbred strains and are less viable diploid males.

The present paper is concerned with sex determination in *Telenomus fariai*, a parasite of the eggs of the "barbeiro" bug, *Triatoma megista* (Burm). In contrast to Habrobracon, close inbreeding is the rule in *Telenomus fariai*. In Telenomus, daughters and sons of the same mother copulate before leaving the eggshell of the host. Despite the brother-sister mating, diploid males are unknown. This difference in the behavior of Habrobracon and Telenomus is in close relation with the remarkable cytological mechanism present in the latter form and described in the following pages.

It is a pleasure to acknowledge our obligation to PROFESSOR THEODOSIUS DOBZHANSKY for his suggestions and his help in the preparation of the English manuscript.

MATERIAL AND METHOD

Telenomus fariai, a representative of Celionidae, has been described by COSTA LIMA (1927). In another paper (1928), COSTA LIMA has pointed out the existence in this species of two classes of males, smaller ones which come from mothers which have been fertilized, and larger ones produced by virgin mothers. COSTA LIMA conjectured that the former are diploid and the latter haploid. TOLEDO PIZA (1929) has discussed the conjecture of COSTA LIMA, without, however, adducing any new facts. The present work was started originally with the intention of clarifying this problem of male dimorphism. Thanks to the kindness of DR. COSTA LIMA, we received two years ago some living material of Telenomus, which has been bred in our laboratory since then, and with which all our work has been done.

A detailed description of the biology of *Telenomus fariai* will be published elsewhere. The points essential for our present purposes are as follows. A fertilized female deposits a varying number of eggs (about seven or eight on the

average) into a single egg of Triatoma. The offspring of fertilized females consists of daughters and sons, always with a predominance of the former. Difficulties have been experienced in attempts to obtain virgin females; females which emerge from the eggs of the host proved to be nearly always impregnated, even if isolated immediately after their emergence. After much experience we have come to the conclusion that the only way to obtain virgins is to break the eggshell of the host and to isolate the females as soon as the latter hatch from the pupae. Indeed, wasps hatching from their pupae do not leave the eggshell of the host immediately, but remain inside for some time, move freely, and evidently copulate before emergence.

We can confirm the observation of COSTA LIMA (1928) that sons of virgin females are larger on the average than those of females which have been fertilized. The genetic constitution of both types of males, however, is the same; both always have ten chromosomes. The simple explanation of the dimorphism is as follows. Male larvae develop more slowly than the female ones; the latter consume more food and cause starvation of their more slowly growing masculine sibs. The offspring of virgin females consists of male larvae only; here the male larvae need not suffer from the competition of females and, though developing slowly, consume sufficient food to permit them to attain a greater average size than is attained by the sons of fertilized females. It must be noted, however, that while during the larval stage the growth of male larvae is slower than that of female larvae, during the pupal stage there occurs an inversion, and the males hatch from the pupae inside the egg of the host before their sisters. Although, as stated above, fertilized females produce fewer sons than daughters, a single male is sufficient to insure insemination of most or of all of his sisters inside the eggshell of the host. *Telenomus fariai* reproduces normally by brother-sister mating.

Acetic orcein, acetocarmine, and the liquids of Duboscq-Brasil and Gilson-Petrunkewitsch have been used as fixatives, acetocarmine, acetic orcein, iron haemotoxylin, and Feulgen as stains. Testes of adult males contain only spermatozoa; to study the early stages of the spermatogenesis, larvae and pupae have been used. The preparations were made by various methods: dissection of testes and ovaries, inclusion in paraffin, colorations in toto of the gonads, etc.

SOMATIC CHROMOSOMES

Figures 1 to 3 show the metaphase plates of somatic mitoses (chiefly of the nerve cells of larvae) in the females. The chromosome number is 20. A closer inspection discloses an important fact—namely, that the largest pair of V-shaped chromosomes consists of unequal partners. One of the V's is always equal-armed and the other is unequal-armed. The longer arm of the asymmetrical V is as long as either arm of the symmetrical V. This difference is always clear, although it is relatively more pronounced at the beginning of the metaphase when the chromosomes are less condensed (compare figures 1 and 2 with figure 3). There are two or three more pairs of V-shaped chromosomes and one pair of J-shaped ones, but no dimorphism is noticeable in these nor in the remaining rod-shaped ones.

Regardless of whether the offspring of fertilized or of virgin mothers is concerned, the somatic cells of the males have ten chromosomes (fig. 4–7). One of the ten is a large V-shaped chromosome, similar to the large equal-armed V in the females. In some more or less exceptional somatic cells chromosome numbers greater than ten are encountered; for example, figure 8 shows a cell with 18 chromosomes. This variation in chromosome number in somatic cells in Hymenoptera is well known and has been mistakenly interpreted as due to chromosome fragmentation. We know that not fragmentation but reduplication of some of the chromosomes owing to non-disjunction (polysomy) is here involved. Despite the variations, the chromosome number in the male is given as ten because (1) ten is the minimum and the most frequently observed number, and (2) the spermatogonia have always ten chromosomes. Some variations of the chromosome number have been observed also in the somatic cells of females.

SPERMATOGENESIS

The spermatogenesis in Hymenoptera has been studied by MEVES, DONCASTER, MARK and COFELAND, MEVES and DUESBERG, LAMS, GRANATA, ARMBRUSTER, NACHTSHEIM, WIEMAN, PATTERSON, TORVIK-GREB, and others (a review in SCHRADER and HUGHES-SCHRADER 1931). All these authors agree in general that one or both meiotic divisions are abortive. There are two variant types of spermatogenesis. The first, found in the honey bee, is characterized by the first division being abortive and resulting in the expulsion of a cytoplasmic bud without a nucleus, while a second division results in an equal division of the nucleus and a very unequal division of the cytoplasm, with the smaller of the two cells degenerating, and a single primary spermatocyte giving rise to a single spermatozoon. The second type, more common than the first and found for example in ants, entails also an abortive first division with an expulsion of a cytoplasmic bud without a nucleus, while the second division is equal, so that a single primary spermatocyte gives rise to two spermatozoa. *Telenomus fariai* represents a third variant type of spermatogenesis.

Figure 9 shows the chromosomes of an early spermatogonial mitosis; ten chromosomes may be counted, one of which is a large V with equal arms, similar to that found in male somatic cells. Figure 10 shows a mitosis of a very special kind observed solely in spermatogonia. The large equal-armed V-shaped chromosome seems to undergo an unequal division. This division results in formation of an equal-armed V which passes to one pole and an unequal-armed V which passes to the other pole. A notable fact is that one of the daughter cells derived from this division is much smaller than the other (fig. 11), does not divide further, and degenerates (fig. 12). Although it is not possible to verify by direct observation that the degenerating cell is the one which receives the large equal-armed V-shaped chromosome, this is made certain by the fact that in the spermatocytes the large V-shaped chromosome is always unequal-armed (fig. 13–15). The presence of small degenerating cells is always evident in young testes and may be observed also in older ones (fig. 16).

The unequal spermatogonial division is followed by a growth period (fig. 17) and the appearance of first spermatocytes, which may be distinguished

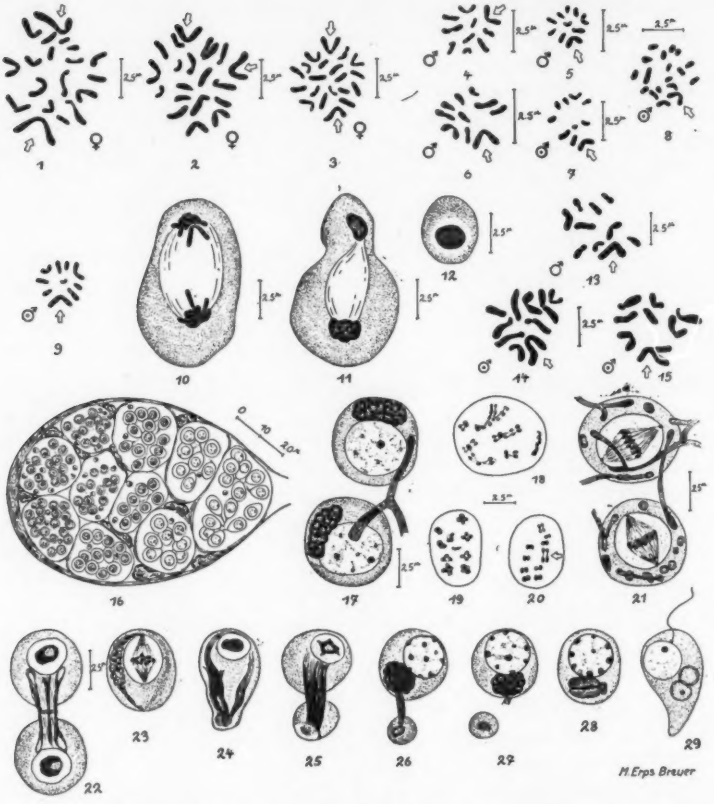

FIG. 1–3.—Nerve cells of ♀ ♀; arrows indicate the pair of V-shaped chromosomes consisting of unequal partners (X and Y). FIG. 4–6.—Nerve cells of ♂ ♂; arrows indicate the X chromosome. FIG. 7.—Somatic cell of a ♂. FIG. 8.—Somatic cell of a ♂ with 18 chromosomes. FIG. 9.—Spermatogonium of a ♂; the arrow indicates the X chromosome. FIG. 10–12.—Spermatogonia of ♂ ♂ undergoing the unequal mitosis. FIG. 13.—A spermatocyte I of a ♂; the arrow indicates a V-shaped chromosome with unequal arms (Y). FIG. 14, 15.—Spermatocytes I of ♂ ♂; arrows show the Y chromosomes. FIG. 16.—A section of a testis of a ♂, showing on the left spermatogonia and small degenerating cells and on the right spermatocytes and some degenerating cells. FIG. 17.—Growing spermatocytes I (♂). FIG. 18–20.—Prophase of the first division showing meiosis-like configurations of chromosomes. In Fig. 20 the arrow indicates the large V-shaped chromosome. Fig. 21.—Metaphase I. Fig. 22.—Telophase I (♂). Fig. 23.—Abortive division II in a ♂. Fig. 24.—Spermatocyte II of a ♂, beginning of the extrusion of cytoplasmic bud. FIG. 25–27.—Elimination of a cytoplasmic bud in a ♂. FIG. 28, 29.—First stages of spermiogenesis. ♂ ♂—sons of fertilized ♀ ♀; ♂ ♂—sons of virgin ♀ ♀. FIG. 1–4, 10–12.—Acetic orcein; 13–15.—Acetocarmine. FIG. 5–9.—Gilson-Petrunkewitsch, total preparations, Feulgen stain. FIG. 16–29.—Duboscq-Brasil, sections, iron haematoxylin.

from the spermatogonia by their large size. In contrast to other Hymenoptera so far studied, the first division of the spermatocytes in Telenomus does not result in expulsion of a cytoplasmic bud without a nucleus. Figures 18 to 20 show a prophase of this division in which the chromosomes assume shapes resembling meiotic bivalents. This, however, is an appearance only; the first metaphase shows ten chromosomes and is followed by a normal cell division and formation of two equal second spermatocytes (fig. 21, 22). The second division, on the contrary, is abortive and does not result in a nuclear division. A spindle is formed with a single centriole (fig. 23), an irregular metaphase plate appears in which one can see a large V-shaped unequal-armed chromosome, whereupon the nucleus reverts to the resting stage. A constriction appears in the cytoplasm on the side of the cell opposite to that containing the single nucleus, separating a globule which remains for some time attached to the cell from which it arose (fig. 24–27). Hence, in Telenomus, as in ants, two spermatozoa arise from each first spermatocyte, but the abortive division is the second and not the first. The process of spermiogenesis begins very soon after the expulsion of the cytoplasmic globule (fig. 28, 29). This is a further proof that the abortive division is the second and not the first. The behavior of the chondrioma ("Nebenkern") is easily observable and presents no peculiarities; here as in other Hymenoptera it resolves itself into elements which become arranged along the spindle (fig. 22) and which persist in the cytoplasmic bridges which join the cells together (fig. 17, 21).

OOGENESIS

The chromosome behavior in oogenesis is on the whole normal, except that the oocyte before the maturation divisions shows an intranuclear spindle which is sometimes perfectly regular (fig. 30, 30a), sometimes less regular, and sometimes even multipolar. This spindle regresses, and the nucleus reverts to its condensed state in which it persists for a long time while the oocyte grows (fig. 31). The meiotic divisions take place after the entrance of the spermatozoon into the egg (fig. 32–37). Four nuclei are formed (fig. 35), of which two fuse (fig. 36, 37) and eventually give rise to the so-called polar nuclei which become the trophamnion. Of the two remaining nuclei, one degenerates and the other (the female pronucleus) fuses with the nucleus of the spermatozoon (the male pronucleus). A study of the embryogenesis shows that in Telenomus, in contrast to what has been seen in related forms, there seems to be no visible differentiation of the cells of the germ track.

DISCUSSION

The classical work of BRIDGES has shown that in Drosophila the sex is determined by the relation between the number of X chromosomes and that of sets of autosomes. The situation in Hymenoptera does not fit this scheme, because here it seems as if the absolute number of chromosomes decides the sex of the offspring. The notable work of WHITING (1935a, 1935b) and of his students, especially of BOSTIAN (1934), however, has thrown a new light on this problem. In the parasitic wasp *Habrobracon juglandis* it has been shown that a female is always heterozygous (XY), while males are of two sorts (X and Y). Diploid homozygotes (XX and YY) are less viable diploid males. A similar scheme fits

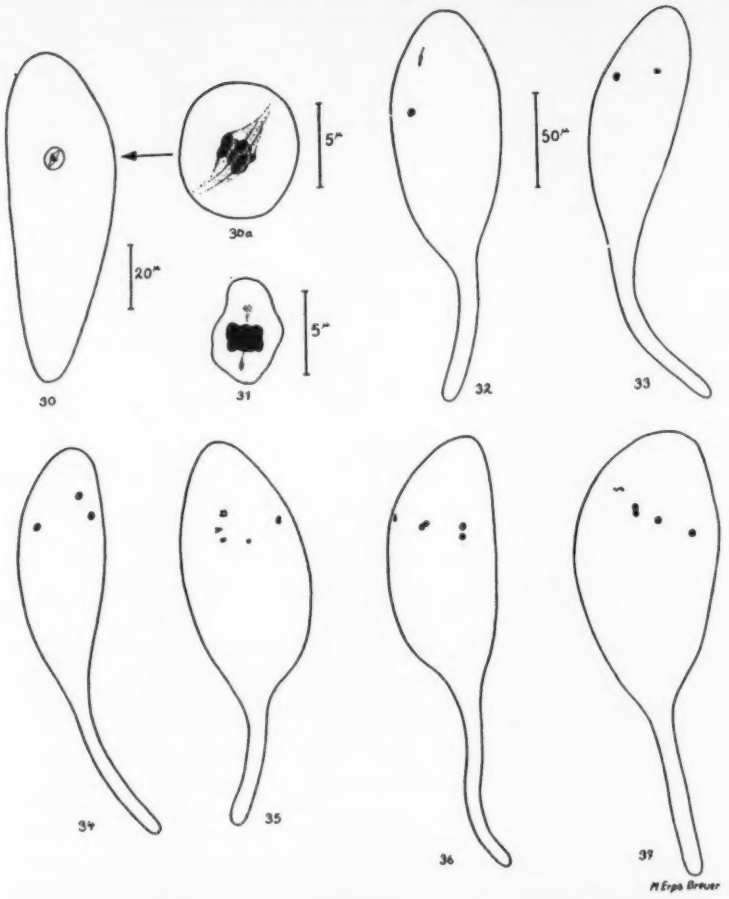

FIG. 30.—An abortive mitosis in a growing oocyte; a detail of the nucleus in Fig. 30a. FIG. 31.—A nucleus of an oocyte before fertilization. FIG. 32.—Fertilization. FIG. 33.—Fertilized oocytes; head of the spermatozoon transformed in ♂ pronucleus. FIG. 34, 35.—Meiotic divisions in oocytes. FIG. 36, 37.—Fusion of two polar nuclei. Gilson-Petrunkewitsch, total preparations, Feulgen.

also the case of another parasitic wasp, *Pteromalus puparum* studied by DOZORCEVA (1936a, 1936b). More recently, WHITING (1939, 1940a, 1943) and BOSTIAN (1939) came to the conclusion that in Habrobracon there is a multiplicity of alleles of the sex-determining locus in the X chromosome, and, therefore, many kinds of X chromosomes X^1, X^2, X^3, etc.) occur in the species. Several kinds of diploid females and several kinds of haploid males are encountered. Indeed, with only six kinds of X chromosomes (X^1–X^6), 15 genetically distinct kinds of females may be produced. In a species in which outbreeding is the rule, there is a reasonable probability that a male which fer-

tilizes a female will have a different kind of X chromosome than the female has, and, therefore, no diploids having the same X chromosome twice will be produced. These diploids (X^1X^1, X^2X^2, etc.) are known to be less viable diploid males. Evidently, the more sex-determining alleles a species has, the smaller the probability of the appearance of diploid males with outbreeding. Inbreeding, however, is followed by the production of diploid males. WHITING (1943) regards the different sex-determining chromosomes of Habrobracon as differing from each other in "differential chromosome segments" rather than in the allelic state of a single sex-determining locus, as thought earlier.

The situation known to exist in Habrobracon helps us to understand the biological meaning of some of the peculiarities of the chromosomal mechanism found in Telenomus. In contrast to Habrobracon, Telenomus reproduces mostly if not exclusively by brother-sister mating. According to the Habrobracon scheme, we might expect that 50 percent of diploid zygotes of Telenomus would develop into less viable diploid males, which are useless to the species. Natural selection could hardly permit a species so wasteful to survive. In reality, however, Telenomus seems to have developed the following mechanism which makes brother-sister mating compatible with non-production of diploid males. The female has a pair of large V-shaped chromosomes, one member of which is equal-armed and the other unequal-armed. We may call these chromosomes respectively X and Y. The somatic cells of the male and the earliest spermatogonia have always an equal-armed V-shaped chromosome (X), and this regardless of whether the male is a son of a fertilized or of an unfertilized female. We are forced to conclude that the process of maturation of the eggs is selective, in the sense that the functioning female pronucleus always carries the X chromosome. If so, the XY constitution of the female presupposes that the spermatozoa always carry a Y chromosome. How is it possible, however, for males which carry an X chromosome to produce spermatozoa with Y chromosome? The answer to this is apparently furnished by the unequal division of the early spermatogonia described above. These spermatogonia divide in two cells, one of which carries an equal-armed V (X) and the other an unequal-armed V (Y). It seems that during this process the X chromosome suffers an unequal division into two chromosomes, one of which is X and the other $X-a=Y$. The cells which receive the X chromosome degenerate and give the small spherules which eventually disappear. The cells which receive the Y are transformed into spermatocytes, undergo the further evolution described above, and give rise to functional spermatozoa, all of which carry a Y chromosome. Early spermatogonia show, therefore, an equal-armed V, and the spermatocytes an unequal-armed V. Owing to the technical difficulties, it has not been possible to demonstrate by direct observation that the female pronucleus always carries an X chromosome; this is inferred from the constitution of the somatic cells and of the early spermatogonia of the males.

SUMMARY

Fertilized females of the parasitic wasp *Telenomus fariai* produce offspring of both sexes, the males being small. Unfertilized females give rise to males only, but these males are large. The chromosome number in females is 20,

and in males, regardless of their origin, 10. The small size of sons of fertilized females is due to male larvae developing more slowly than their female sibs and consequently suffering from competition with the latter for food.

The female chromosome group contains a pair of large V-shaped chromosomes which consist of unequal partners: one chromosome is equal-armed and the other unequal-armed. The former is called the X and the latter the Y chromosome. Somatic cells and the early spermatogonia of the males have always the X chromosome. The spermatogonia suffer an unequal division, during which the X chromosome divides in such a way that an equal-armed V (X) passes to one and an unequal-armed V (Y) passes to the other pole. The cells which receive the X chromosome degenerate, and those receiving the Y become spermatocytes and are transformed eventually into Y-bearing spermatozoa. It is inferred that the Y chromosome differs from the X by the absence of a section of one of the two limbs ($X - a = Y$). Oogenesis is normal; it is supposed that the female pronucleus has always an X chromosome. This remarkable chromosome behavior is presumably connected with the fact that *Telenomus fariai* reproduces normally by brother-sister mating. In other parasitic Hymenoptera, such as Habrobracon, inbreeding leads to production of some only slightly viable diploid males, which have never been observed in Telenomus.

The spermatogenesis in Telenomus differs from that in other Hymenoptera in that the first division of the spermatocyte is normal and gives rise to two second spermatocytes. The second division is abortive and leads to the expulsion of the enucleated bud of the cytoplasm.

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STUDIES ON THE EFFECT OF X CHROMOSOME INVERSIONS ON CROSSING OVER IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER

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INTRODUCTION

ALL present theories of crossing over tacitly assume that events in one tetrad (at least as far as crossing over is concerned) are independent of those occurring in the other tetrads of the cell. Yet as long ago as 1919 STURTEVANT suspected that the presence of a heterozygous inversion in one chromosome pair increases crossing over in a non-homologous pair of chromosomes. The effect was definitely established by SCHULTZ and REDFIELD (MORGAN, BRIDGES) and SCHULTZ 1932, 1933), who showed that in *Drosophila melanogaster* heterozygous inversions in the first and/or second chromosomes increase crossing over in the third chromosome and that heterozygous inversions in the first and/or third chromosomes increase crossing over in the second chromosome, and by GLASS (1933) who found that an inversion in the second chromosome increased crossing over in the third chromosome (this observation was independent of that of SCHULTZ and REDFIELD). Subsequently STEINBERG (1936) showed that crossing over was increased in the first chromosome in the presence of heterozygous inversions in the second and/or third chromosomes.

These experiments all involved an increase in crossing over in a tetrad which was not itself heterozygous for an inversion. STURTEVANT's original observation was on a tetrad which was itself heterozygous for an inversion, although the increase in crossing over took place in an uninverted segment. He found that crossing over between the second chromosome mutants purple and curved, which was reduced to about one percent in the presence of the inversions carried in the second chromosome of the Nova Scotia stock, was increased to about 20 percent (that is, approximately the standard value) in the simultaneous presence of an inversion in the third chromosome. STURTEVANT's observations were supported by GLASS's report in 1933 that crossing over within the Plum inversion in the second chromosome was increased in the presence of an heterozygous inversion in the third chromosome. SIDEROW, SOKOLOW, and TROFIMOW (1936) in making use of GLASS's observation for other purposes showed that heterozygous inversions in the second and third chromosomes increase crossing over in the X chromosome when it also is heterozygous for an inversion. These data are of particular interest because the regions of the X chromosome studied by these authors are similar to those studied by STEINBERG (1936) and may be compared with them. They will be discussed in this connection in a later section of this paper. The observations on *Drosophila melanogaster* were confirmed for *Drosophila pseudoobscura* by MACKNIGHT

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(1937), who found that heterozygous inversions in the second and third chromosomes increased crossing over in the X chromosomes when they (the X chromosomes) were heterozygous for inversions in each of the arms.

SCHULTZ, MATHER, and STEINBERG and WHITE have each advanced working hypotheses to explain the above observations. SCHULTZ'S (MORGAN, BRIDGES, and SCHULTZ 1935) hypothesis involves the assumptions (a) that crossing over is a function of the twisting of the chromosomes about each other, (b) that the presence of an inversion in the heterozygous condition interferes with somatic pairing in the last premeiotic division of the pair of chromosomes bearing the inversion, and (c) that this will lead to "an increased likelihood of overlying contacts or twists near the spindle fiber of that pair of chromosomes when they do synapse in prophase; and this overlap will be correlated with a similar occurrence in another pair," thus resulting in increased crossing over in the latter pair. From this hypothesis it follows that the degree of disturbance in pairing is the factor which determines the extent of the interchromosomal effect of an inversion on crossing over. Hence one would expect to find a relationship between the length and position of an inversion in the chromosome on the one hand and the magnitude of its interchromosomal effect on the other.

MATHER'S (1936) hypothesis to explain the interchromosomal effect of inversions on crossing over involves the assumption that the total number of chiasmata in any given cell under fixed conditions is limited and that the tetrads within the nucleus compete for this limited number. If, therefore, one tetrad has fewer chiasmata than usual, the other tetrads in the cell may be expected to have more chiasmata than usual. On the basis of this hypothesis one would expect that those inversions which interfere most with crossing over within the tetrad bearing them would have the greatest interchromosomal effect on crossing over. It might be well to point out here that this hypothesis cannot explain the data involving an increase in crossing over caused by heterozygous inversions in a non-homologous tetrad which is also heterozygous for an inversion (see discussion of STURTEVANT, GLASS, *et al.* above). Although crossing over was measured in only one of the tetrads concerned, it must be clear that crossing over was increased in both tetrads. This necessitates an increase in the total number of chiasmata in the cell and therefore does not conform to MATHER'S hypothesis which involves competition among the tetrads for a fixed number of chiasmata.

The hypothesis proposed by STEINBERG and WHITE (1939) postulates that the interchromosomal effect of inversions on crossing over is physiological and not mechanical in nature. This was based on two points (a) the fact that many inversions are known to have physiological effects (position effect) and (b) the fact that the magnitude of the effect of an inversion on crossing over in a given non-homologous chromosome is directly proportional to the relative amount of the total chromatin of the cell contained in the affected chromosome (STEINBERG 1937). On the basis of this hypothesis no correlation between the size or position of the inversion in the chromosome and the magnitude of its effect would be expected.

The experiments reported below were designed to test these hypotheses.

MATERIALS AND METHODS

Crossing over was measured in the third chromosome by means of the "rucuca" complex of recessive markers. There follows a brief description of the mutants used; a more detailed description will be found in Drosophila Information Service No. 9. The wild type stock used was a strain of Oregon-R maintained in mass culture.

SYMBOL	NAME	LOCATION ON STANDARD 3RD CHROMOSOME MAP	DESCRIPTION
ru	roughoid	0.0	Eye rough and small
h	hairy	26.5	Extra hairs on wings
th	thread	43.2	Arista thread-like
st	scarlet	44.0	Eye-color bright scarlet
cu	curled	50.0	Wings upcurled
sr	stripe	62.0	Dark dorsal stripe
e^s	sooty	70.7	Body-color dark
ca	claret	100.7	Eye-color ruby

The crossover regions were numbered as follows:—

ru [1] *h* [2] *th* [3] *st* [4] *cu* [5] *sr* [6] e^s [7] *ca*

and will be referred to as such in the text unless otherwise indicated.

The effects of twelve different X chromosome inversions were tested. Table 1 lists these inversions, giving the cytological and where known the genetic positions of the left and right breaks.

TABLE 1

Description of the X Chromosome inversions used.

INVERSION	SALIVARY GLAND DATA		GENETIC DATA		REFERENCE
	LEFT BREAK	RIGHT BREAK	LEFT BREAK	RIGHT BREAK	
bb^{Df}	4D1–2	19F Df in 20 C-D	between *rb* and *rg*	between *car* and sp-a	STURTEVANT and BEADLE (1936) Salivaries—SUTTON Unpublished
dl-49	4D7-4E1	11F2–11F4	between *rb* and *cv*	between *fw* and *g*	STURTEVANT and BEADLE (1936) Salivaries—HOOVER (1938)
ClB	4A5	17A6	between *sc* and *bi*	between *sy* and *fu*	STURTEVANT and BEADLE (1936) Salivaries—HOOVER (1938)
sc^7	1B4	5D5	between *sc* and *sv*	between *cv* and *ct*	STURTEVANT and BEADLE (1936) Salivaries—SUTTON Unpublished
sc^8	1B2	20B+	between *ac* and *sc*	between *bb* and sp-a	STURTEVANT and BEADLE (1936) Salivaries—D.I.S. 9
y^4	1A	17	left of *y*	between *fu* and *da*	MULLER and PROKOFYEVA (1934) Salivaries—D.I.S.9
AM	8C17–8D1	16E2–16E3	near *lz*	between *B* and *Bx*	STONE and THOMAS (1935) Salivaries—HOOVER (1938)
sc^4	1B4	19F1–2	between *sc* and *sv*	between *car* and *bb*	STURTEVANT and BEADLE (1936) Salivaries—SUTTON Unpublished
AB	about 9F	13F1	near *v*	between *g* and *sd*	STONE and THOMAS (1935)
A99B	1D3–1E1	19D–19E	not determined		D.I.S. 12
sc^{260-14}	1B1.2–1B3.4	11D3–11D8	not determined		SUTTON (1943b)
B^{263-47}	16A1.2–16A4	beyond 20A1.2	not determined		SUTTON (1943a)

The plan of the experiments in all cases except those involving the *ClB* and *dl-49* inversions was as follows:—

P_1:-3, 4 or 5 rucuca ♀ ♀ ×4 or 5 Inversion-bearing ♂♂.

B.C.:-3 F_1 ♀ ♀ heterozygous for the inversion and rucuca×4 rucuca ♂♂.

In the case of the *ClB* and *dl-49* inversions the P_1 consisted of inversion bearing ♀ ♀ mated to rucuca ♂♂; otherwise the crosses were the same as in the other experiments. *ClB* ♀ ♀ were used for the obvious reason that no *ClB* ♂♂ survive; ♀ ♀ heterozygous for *dl*-49 were used because of the low fertility exhibited by the *dl-49* ♂♂ of this stock.

In those cases in which the inverted chromosome carried markers which might interfere with the classification of any of the rucuca characters, only backcross flies not showing the markers were classified. Thus in the case of the sc^4 inversion which carries yellow, only the ♀ ♀ and non-sc^4 ♂♂ were classified; in the case of the y^4 inversion only the ♀ ♀ were classified; because the sc^7 and sc^8 inversions carried apricot, only the ♀ ♀ and non-apricot ♂♂ were classified; in the experiment involving the *dl-49* inversion only ♂♂ not carrying the *dl-49* inversion were classified because it is difficult to distinguish between *Hw* (Hairy-wing) carried by the *dl-49* inversion and *h* of the rucuca complex; in the *ClB* experiment the ♂♂ and the non-*ClB* ♀ ♀ were classified because of uncertainty in the classification of some eye colors in the presence of Bar. In all other cases all backcross offspring were classified.

In all cases the backcross generation was classified for nine days, including the first day of eclosion.

Throughout these experiments the standard cornmeal, agar, molasses food medium reinforced with dried brewer's yeast was used. All crosses were raised at 25°±0.2°C.

Further details of technique will be given in the appropriate places in the text.

DATA

Because the experiments were done in three groups to test different questions, and also for the sake of clarity, they will be presented in chronological order. The first set of data involved the following six inversions: bb^{D1}, *ClB*, *dl-49*, sc^8, sc^7, and y^4 (table 1). The raw data (except for the combining of the sexes) are given in table 10 at the end of the text. Table 2 shows the crossover values obtained in each region for each of the crosses and the percentage change which these values show with respect to those of the control. Table 3 presents the distribution of the strands among the various types of crossovers obtained and the χ^2 values derived from a comparison of each of the test crosses with the control.

Among the various methods which may be employed to compare control and test crossover values, that of comparing the strand distribution by means of 2×n contingency tables is the most direct, because each strand represents one tetrad and because the strand data are basic to the calculation of crossover frequencies. The χ^2 values listed in table 3 are derived from comparisons of each of the crosses with the control. For these calculations four and five point

TABLE 2

Comparison of test and control crossover values.

INVERSION TESTED	REGION 1 % CROSS-OVER	1 % CHANGE	2 % CROSS-OVER	2 % CHANGE	3 % CROSS-OVER	4 % CROSS-OVER	4 % CHANGE (INCLUDING REGION 3)	5 % CROSS-OVER	5 % CHANGE	6 % CROSS-OVER	6 % CHANGE	7 % CROSS-OVER	7 % CHANGE	MAP LENGTH	% CHANGE	TOTAL
Control (1)	24.8	—	21.1	—	1.0	5.6	—	13.9	—	10.6	—	38.0	—	115.0	—	2419
bb^{Df}	29.7	19.6	22.5	6.6	1.5	9.1	60.6	13.4	−3.6	9.3	−12.3	32.6	−14.2	118.1	2.7	2520
ClB	27.0	8.9	23.5	11.4	0.5	8.1	30.3	18.0	29.5	11.0	3.8	35.3	−7.1	123.4	7.3	2116
dl-49	26.6	7.3	23.8	12.8	0.8	7.8	30.3	16.8	20.9	11.3	6.6	39.0	2.6	126.1	9.7	1585
sc^{8}	24.7	−0.4	26.8	27.0	1.0	11.1	83.3	18.1	30.2	11.6	9.4	35.3	−7.1	128.6	11.8	1610
sc^{7}	27.6	11.3	25.7	21.8	1.1	8.5	45.4	16.6	19.4	12.0	13.2	37.7	−0.8	129.2	12.3	3116
y^{4}	24.9	0.4	24.1	14.2	1.3	14.4	137.8	21.5	54.7	11.4	7.5	35.4	−6.8	133.0	15.7	2711

crossover strands were grouped so that n (number of degrees of freedom) equals 4. In addition, many of the crossover values were compared with the appropriate control values in 2×2 contingency tables.

Five of the six inversions tested showed a significantly different strand distribution from that of the control (Inversions *ClB*, *dl-49*, sc^8, sc^7, and y^4 (table 3)). The deviation from the control values was in the same direction in every case—namely, a reduction in non-crossover strands, little or no change in the single crossover strands, and an increase in multiple crossover strands

TABLE 3

Classification of strands into crossover classes.

INVERSION TESTED	ZEROS		SINGLES		DOUBLES		TRIPLES		QUADS		QUINTS		TOTAL	χ^2 (AGAINST CONTROL)
	N	%	N	%	N	%	N	%	N	%	N	%		
Control (1)	565	23.5	1107	45.8	576	23.8	160	6.6	11	0.5	0	0.0	2419	—
bb^{D1}	600	23.8	1078	42.8	649	25.8	173	6.9	19	0.8	1	0.0+	2520	6.9
ClB	453	21.4	893	42.2	610	28.8	144	6.8	15	0.7	1	0.0+	2116	17.8
dl-49	310	19.6	697	44.0	452	28.5	106	6.7	20	1.3	0	0.0	1585	23.3
sc^8	322	20.0	672	41.7	476	29.6	125	7.8	14	0.9	1	0.0+	1610	26.0
sc^7	567	18.2	1358	43.6	929	29.8	240	7.7	22	0.7	0	0.0	3116	40.9
y^4	495	18.3	1124	41.5	823	30.4	243	9.0	23	0.8	3	0.1	2711	55.1

When n=4; P=0.05 when χ^2=9.5; P=0.01 when χ^2=13.3

resulting in an increase in crossing over. The sixth inversion (bb^{D1}) did not show a significantly different strand distribution from that of the controls, P>0.1.

The χ^2 values listed in table 3 vary from 6.9 to 51.1 in more or less discrete steps which follow the same order of increasing magnitude as do the corresponding total map lengths listed in table 2. This relationship is to be expected, since the crossover values are derived from the strand data.

The data were examined to find whether or not any relationship existed between the size and position of the inversions and the effect on crossing over in the third chromosome. There is no correlation between size (either genetic or cytological) of the inversion and the magnitude of its interchromosomal effect. For example, the relatively short sc^7 inversion has a considerable effect on crossing over in the third chromosome, while the relatively long bb^{D1} inversion has no effect; also among those inversions with an effect, the short sc^7 and the long y^4 inversion each has a great effect on crossing over, while the long *ClB* and the short *dl-49* each has relatively little effect (tables 1, 2, 3). The only physical feature of the inversions which shows any correlation with the magnitude of the interchromosomal effect on crossing over is the position of the left break of the inversion. The closer this break lies to the left end of the chromosome the greater the magnitude of its interchromosomal effect on crossing over (tables 1, 2, 3). While no acceptable explanation of why such a relationship should exist was apparent, it was deemed advisable to test this relationship further. Accordingly, two other inversions, *AM* with its left break relatively far from the left end and sc^4 with its left break relatively close to the left end (table 1) were tested.

If the relationship between the position of the left end of the inversion and the magnitude of the interchromosomal effect on crossing over is real, it is to be expected that the *AM* inversion would have no effect on crossing over in the third chromosome, while the *sc*^{4} inversion would have a very large effect. The raw data are listed in table 10, the crossover values and their relative change with regard to the controls are in table 4, and the strand analysis is in table 5. The χ^2 values derived from a comparison of the strand distribution values of each of the test crosses with those for the control are 10.9 for the *AM* inversion and 11.1 for the *sc*^{4} inversion; with four degrees of freedom P falls between 0.05 and 0.02 ($\chi^2 = 11.7$ at $P = 0.02$). This indicates that the strand distributions may be different from those of the control, but nevertheless there is more than one chance in 50 in either case that the value of χ^2 derived will be exceeded. Comparison of the crossover values of each of the test crosses with those of the controls shows that the values are statistically identical in all cases. (Region 7 is not considered, because the control value for some unexplained reason is abnormally high as compared with the standard value (38.0 as compared to 30.0)). Because of this, the authors feel inclined to consider the strand distributions of the controls and tests to be the same. However, for the purpose of our present discussion the question of primary importance is not whether the strand distributions derived in the presence of the *AM* and *sc*^{4} inversions are the same as or different from those of the control, but "how do the values derived in the presence of these inversions compare with each other?" The strand distributions are statistically the same ($\chi^2 = 5.8$, $n = 4$, hence $P > 0.2$). χ^2 comparisons of *AM* and *sc*^{4} in each of the several crossover regions have not been made because (a) in all except regions 3–4 (regions 3 and 4 have been combined for purposes of statistical treatment because region 3 is so short) and 5, the crossover values are so close as to be obviously the same, (b) *sc*^{4} was compared with the control in region 3–4 and found to be the same ($P > 0.2$) and hence would also be the same as *AM*, and (c) *AM* was compared with the control in region 5 and found to be the same ($P > 0.2$) and therefore would not differ from *sc*^{4} (see the crossover values in table 4). Clearly these data do not conform to the original observation that the position of the left break of the inversion is related to the magnitude of the interchromosomal effect of the inversion on crossing over. No new physical relationship between the nature of the inversion and its interchromosomal effect was derived. It was felt therefore that further tests were desirable before any definite conclusions were drawn.

In order to simplify the technical problem of classification of the flies, a comparison of the individual crossover regions with the appropriate controls was made to see which regions were affected and which were not. Region 7 is of no value in these considerations because of its length, which allows for undetected crossovers, and also because of the abnormally high crossover value given by the controls. Region 1 is also of little value because of its length (26.5 units on the standard map); however, it should be noted in passing that the values obtained in region 1 in the presence of *AM* and *sc*^{7} give a P value of between 0.02 and 0.05 when compared with the control and that the value

TABLE 4

Comparison of crossover values (in presence of AM and sc[4]) with control values.

INVERSION TESTED	REGION 1		2		3	4		5		6		7		MAP LENGTH	% CHANGE	TOTAL
	% CROSS-OVER	% CHANGE	% CROSS-OVER	% CHANGE	% CROSS-OVER	% CROSS-OVER	% CHANGE (INCLUDING REGION 3)	% CROSS-OVER	% CHANGE	% CROSS-OVER	% CHANGE	% CROSS-OVER	% CHANGE			
Control (1)	24.8	—	21.1	—	1.0	5.6	—	13.9	—	10.6	—	38.0	—	115.0	—	2419
AM	27.9	12.5	21.9	3.8	0.7	5.4	−7.6	15.3	10.1	10.4	−1.9	34.7	−8.7	116.3	1.1	1408
sc[4]	25.4	2.4	22.2	5.2	0.5	7.1	15.1	13.8	−0.7	11.4	7.5	33.6	−11.5	114.0	−0.9	2006

derived in the presence of bb^{Df} gives a P value of much less than 0.01. In region 6 no value differed significantly from that of the controls. Region 3 is so short that small differences would be difficult to detect, and it therefore is not very useful. Thus, the only regions which give satisfactory information are regions 2, 3 and 4, and 5.

TABLE 5

Classification of strands into crossover classes.

INVERSION TESTED	ZEROS		SINGLES		DOUBLES		TRIPLES		QUADS		TOTAL	χ^2 (AGAINST CONTROL)
	N	%	N	%	N	%	N	%	N	%		
Control (1)	565	23.5	1107	45.8	576	23.8	160	6.6	11	0.5	2419	
AM	326	23.2	609	43.3	395	28.1	74	5.3	4	0.3	1408	10.9
sc^4	463	23.1	905	45.1	536	26.7	97	4.8	5	0.2	2006	11.1

When n = 4; P = 0.05 when $\chi^2 = 9.5$; P = 0.01 when $\chi^2 = 13.3$.

Four additional X chromosome inversions were tested—namely B^{263-47}, *A99b*, sc^{260-14} and *AB* (table 1). Although the rucuca stock was used as in the previous eight experiments, only *h*, *th*, *cu*, and *sr* were followed. The crossover regions and their equivalents in the earlier experiments are as follows:

New region	Old region	Markers
1	2	*h-th*
2	3 and 4	*th-cu*
3	5	*cu-sr*

Because more than a year had elapsed since the experiments were begun, a new set of controls was studied. The raw data of these crosses (except for a grouping of the sexes) are listed in table 11. The crossover values and the strand analyses are listed in tables 6 and 7, respectively. (For the purposes of χ^2 analyses the double and triple crossover strands were combined.)

TABLE 6

Comparison of Crossover values.

INVERSION TESTED	REGION 1		REGION 2		REGION 3		MAP LENGTH	% CHANGE	TOTAL
	% CROSSOVER	% CHANGE	% CROSSOVER	% CHANGE	% CROSSOVER	% CHANGE			
Control (2)	21.2	—	6.0	—	12.9	—	40.1	—	3918
B^{263-47}	19.8	−6.6	7.3	21.7	14.6	13.2	41.7	4.2	2124
A99b	21.3	0.5	7.0	16.7	14.9	15.5	43.2	7.7	2145
sc^{260-14}	23.9	12.7	7.2	20.0	14.7	14.0	45.8	14.2	2505
AB	25.1	18.4	9.9	65.0	16.4	27.1	51.4	28.2	2144

TABLE 7

Classification of strands into crossover classes.

INVERSION TESTED	ZEROS		SINGLES		DOUBLES		TRIPLES		TOTAL	χ^2 (AGAINST CONTROL)
	N	%	N	%	N	%	N	%		
Control (2)	2511	64.09	1245	31.77	160	4.08	2	0.05	3918	—
B^{263-47}	1326	62.43	712	33.52	83	3.91	3	0.14	2124	1.3
A99b	1333	62.14	701	32.68	108	5.03	3	0.14	2145	4.4
sc^{356-14}	1492	59.56	882	35.21	128	5.11	3	0.12	2505	14.3
AB	1187	55.36	815	38.01	139	6.48	3	0.14	2144	50.1

When n = 2; P = 0.05 when $\chi^2 = 6.0$; P = 0.01 when $\chi^2 = 9.2$.

By rearranging the data of the first nine crosses on the basis of the four markers followed in the last five crosses, it is possible to obtain direct comparisons. The crossover values of the rearranged data are given in table 8, the strand distributions are given in table 9.

The first and second sets of controls when compared in this way are found to be statistically identical in each of the three crossover regions and also with regard to the strand distribution. Therefore there was no change in the rucuca or Oregon-R stocks during the course of these experiments. Of the four inversions tested two were very short; one (*AB*) is located in the central region of the X chromosome, the other (B^{263-47}) in the proximal region of the X chromosome. The former had a marked effect on crossing over in the third chromosome, the latter had none. The remaining two inversions were relatively long; one (*A99b*) includes the entire active region of the X chromosome, the other

TABLE 8

*Comparison of test crossover values with control values.**

REGION		CONTROL (1)	SC^4	AM	BB^{Df}	DL-49	CLB	SC^7	SC^8	Y^4
1	% crossover	21.1	22.2	21.9	22.5	23.8	23.5	25.7	26.8	24.0
	% change	—	5.2	3.8	6.6	12.8	11.4	21.8	27.0	18.5
2	% crossover	6.5	7.5	6.1	9.6	8.5	8.6	9.1	11.9	15.3
	% change	—	15.4	−6.2	47.7	30.8	32.3	40.0	83.1	135.4
3	% crossover	13.9	13.8	15.3	13.4	16.8	18.0	16.6	18.1	21.5
	% change	—	−0.7	10.1	−3.6	20.9	29.5	19.4	30.2	54.7
	Map length	41.5	43.5	43.3	45.6	49.1	50.1	51.4	56.8	60.8
	% change	—	4.8	4.3	9.9	18.3	20.7	23.9	36.9	46.5

* Note new region 1 = old region 2; new region 2 = old regions 3 and 4; new region 3 = old region 5.

In recasting data, crossover strands involving regions 3 and 4 simultaneously were treated as non-crossovers in new region 2.

TABLE 9

Classification of strands into crossover classes.
(Data of table 10 arranged on basis of three regions only.)

	CONTROL (1)		*sc*[4]		*AM*		*bb*[D1]		*dl-49*		*ClB*		*sc*[7]		*sc*[8]		*y*[4]	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0	1541	63.7	1240	61.8	867	61.6	1506	59.8	905	57.1	1188	56.1	1717	55.1	831	51.6	1346	49.6
1	755	31.2	660	32.9	473	33.6	886	35.1	586	37.0	798	37.7	1198	38.4	637	40.2	1094	40.4
2	119	4.9	105	5.2	68	4.8	123	4.9	91	5.7	128	6.0	197	6.3	129	8.0	259	9.6
3	4	0.17	1	0.06	0	0.00	5	0.20	3	0.19	2	0.10	4	0.12	3	0.19	12	0.44
Total	2419		2006		1408		2520		1585		2116		3116		1610		2711	
χ^2				1.6		2.6		8.8		17.5		26.9		41.8		60.8		114.4

When n = 2; P = 0.05 when $\chi^2 = 6.0$; P = 0.01 when $\chi^2 = 9.2$

(sc^{260-14}) about the distal half. *A99b* had no effect while sc^{260-14} caused a significant increase in crossing over in the third chromosome.

The data derived from these four inversions combined with those derived from the eight previously tested make it abundantly clear that neither the size nor the position with respect to the ends of the chromosome nor the combination of these two morphological features of an inversion are related to the interchromosomal effect of inversions on crossing over.

DISCUSSION

For convenience of discussion two aspects of the problem of the interchromosomal effect of inversions on crossing over may be distinguished: (a) the nature of the effect—that is, the magnitude and distribution of the increased crossing over from region to region of the affected chromosome—and (b) the relationship between the effect (its magnitude and distribution) and the nature of the inversion causing it. The two aspects are of course intimately related, and the one cannot be measured without the other.

All the inversions tested showed the same pattern of effect on crossing over in the third chromosome (that is, when any effect was observed). The greatest increase in crossing over occurred in the region of the centromere and fell off sharply on either side (tables 2 and 4). SCHULTZ and REDFIELD (MORGAN, BRIDGES, and SCHULTZ 1932, 1933) reported the same type of distribution of effect in the second and third chromosomes. They observed no difference in the pattern of effect on the third chromosome of inversions in the first and second chromosomes, nor did they observe any differences between the effect of the *ClB* inversion and the Payne inversions on crossing over in the second chromosome. From all these data it appears that the autosomes always respond in the same way to the interchromosomal stimulus of inversions on crossing over. This is not true of the X chromosome, which shows a different pattern of increase in the presence of the Curly inversions from that which it shows in the presence of the Payne inversions (STEINBERG 1936).

The increase in crossing over observed in the third chromosome is associated with a decrease in non-crossover strands and an increase in single and multiple crossover strands. This relationship is most clearly illustrated in tables 7 and 9 where only the three most affected regions are considered, but masked in tables 3 and 5 where all seven regions are considered, because four of the seven regions are either not affected at all or are affected to only a very slight extent. The increased frequency of multiple crossovers may be the result of reduced interference or may be due to a proportional increase of single and multiple crossovers resulting from an increase in crossing over without any change in interference. If interference has been reduced, the frequency of multiple crossover strands relative to the frequency of single crossover strands should be increased. If, on the other hand, interference has not been affected, the frequency of multiple crossover strands relative to the frequency of single crossover strands should remain unchanged. The changed relationship may not be discernible when all seven regions are considered because of the masking effect of the four unaffected regions; however, if only the three most affected regions are considered it should be easily recognized.

A preliminary survey of the data in table 9 indicated that in all except the cross involving the y^4 inversion the ratio of single crossover to multiple crossover strands was statistically the same. Accordingly a χ^2 test for homogeneity was made in a 2×8 contingency table involving the strand data of the original set of controls and that of seven of the first eight inversions (y^4 being omitted); $\chi^2=8.3$, $n=7$, and $P\approx0.30$; hence there is no change in interference. The strand distribution derived in the presence of the y^4 inversion was tested against the control strand distribution in a 2×2 table; $\chi^2=33.2$, $n=1$, $P<<<0.01$, indicating a considerable change in interference. The change is obviously a reduction, since the frequency of multiple crossover strands relative to single crossover strands is greatly increased. The test for homogeneity when repeated with the y^4 inversion included gave a χ^2 value of 29.0, $n=8$, $P<<0.01$. The remaining four inversions were tested against the second set of controls (table 7) in a 2×5 contingency table; $\chi^2=8.8$, $n=4$, $P>0.05$ but <0.1, indicating no change in interference.

The data published by STEINBERG (1936) involving the effect of the Curly and Payne inversions, singly and combined, on crossing over in the X chromosome were tested in the same manner. In each of the three tests $P<<<0.01$, indicating a marked decrease in interference. This was recognized in the earlier publication where, although no statistical tests were made, it was pointed out that there was a considerable increase in multiple crossover strands and only a slight increase in single crossover strands in the presence of the inversions either singly or combined. SIDEROW, SOKOLOW, and TROFIMOW (1936) studied the effect of inversions in the second and third chromosomes on double crossing over within the inverted portion of X chromosomes heterozygous for an inversion. In each of two experiments (one involving In(1) *sc*^{9} the other In(1) *ClB*) the test cross showed a five-fold increase in crossing over as compared to the control value. This magnitude of increase is the same as that obtained by STEINBERG (1936) and indicates that SIDEROW, SOKOLOW, and TROFIMOW's data also involve a great reduction in interference.

Unfortunately SCHULTZ and REDFIELD's data are not published in a form which would permit an analysis of interference changes in their experiments.

It is clear that the increase in crossing over caused by heterozygous inversions in another pair of chromosomes is realized in two different ways. The first method involves a reduction of non-crossover strands and a concomitant reduction in interference. This type of effect is the only one thus far to be observed in the X chromosome, and it has been observed in the third chromosome only in the presence of the y^4 inversion. The second method involves a general increase in crossing over with no accompanying change in interference. This type of increase in crossing over has been observed in the third chromosome in all cases involving an increase, except that associated with the presence of the y^4 inversion.

It will be noted that a tetrad analysis of the data has not been attempted, although an extensive analysis of this type was made in the senior author's earlier paper (STEINBERG 1936). This is so because the work of HEARNE and HUSKINS (1935), HUSKINS and NEWCOMBE (1941), and of LINDEGREN and LINDEGREN (1937, 1939) cast doubt on one of the basic assumptions involved

in the derivation of the formulae upon which the tetrad analysis is based—namely, that the chromatids which cross over at one level do not influence those which cross over at other levels.

We turn now to a discussion of the data in the light of the three hypotheses advanced to explain the interchromosomal effect of heterozygous inversions on crossing over. SCHULTZ's hypothesis requires a correlation between the degree of disturbance of somatic pairing in the last premeiotic division within the chromosome pair heterozygous for the inversion and the interchromosomal effect of the inversion on crossing over. The only direct way of measuring the degree of disturbance of somatic pairing is of course cytological observation of the last premeiotic division. If SCHULTZ's hypothesis is correct, the disturbance in somatic pairing should affect the pairing not only of non-homologous chromosomes but also that of the chromosome pair heterozygous for the inversion. This disturbance should be reflected in the extent to which crossing over is affected in the chromosome pair heterozygous for the inversion and hence should afford an indirect measurement of the degree of disturbance in somatic pairing caused by heterozygous inversions. Seven of the twelve X chromosome inversions utilized in these experiments were studied by STURTEVANT and BEADLE (1936) with regard to their effect on crossing over within the inverted and the uninverted portions of the chromosomes. STURTEVANT and BEADLE's findings are summarized below.

INVERSION	EFFECT ON CROSSING OVER WITHIN THE INVERTED PORTION	WITHIN THE UNINVERTED PORTION	
		TO LEFT OF INVERSION	TO RIGHT OF INVERSION
sc^7	Decreased	*	Decreased
dl-49	Decreased	Decreased	Decreased
ClB	Probably decreased	Decreased	Decreased
sc^4	Little or possibly no effect	*	*
sc^8	Little or possibly no effect	*	*
bb^{Df}	Little or possibly no effect	Decreased	*
y^4	Little or possibly no effect	*	Decreased

* No uninverted section in which crossing over occurs in normal flies is present, and therefore no tests can be made.

From these data it follows that crossing over is affected by the inversions in the following order of decreasing magnitude: $dl\text{-}49 \geqq ClB > sc^7 > bb^{Df} \geqq y^4 > sc^4 = sc^8$. Presumably the degree of disturbance experienced in somatic pairing in the last premeiotic division would follow the same seriation. However, the relative magnitude of the effect of these inversions on crossing over in the third chromosome follows no such seriation (tables 2, 3, 4, 5, 7, 8). For example, both sc^4 and sc^8 are very long inversions and have little or no effect on crossing over in the X chromosome, yet sc^8 has a considerable effect on crossing over in the third chromosome, while sc^4 has no effect; y^4 is a long inversion and has little or no effect on crossing over in the X chromosome, while *ClB* is of medium

length and reduces crossing over in the X chromosome to a very great extent; nevertheless, the y^4 inversion causes a significantly greater increase in crossing over in the third chromosome than does the *ClB* inversion; on the other hand, although the *ClB* inversion has a much greater effect on crossing over in the X chromosome than does the sc^4 inversion, it has a significantly greater effect on crossing over in the third chromosome. When to this group of seven inversions we add the *AM* and *AB* inversions, which STONE and THOMAS (1935) have shown to cause a considerable reduction in crossing over in the X chromosome, and the *A99b* and sc^{260-14} inversions, which probably have little or no effect, and the B^{263-47} inversion, which probably has a considerable effect on crossing over in the X chromosome, the breakdown of a correlation between the effect of an inversion on crossing over in the X chromosome and its effect on crossing over in the third chromosome becomes complete. From these considerations it follows that SCHULTZ's hypothesis must be abandoned.

MATHER's theory of "competitive pairing" (the name is an unfortunate one, since it has already been used much more appropriately by DOBZHANSKY (1934) to describe his hypothesis (DOBZHANSKY 1931, 1932) concerning the competition between the portions of rearranged chromosomes for pairing with their homologues) requires an inverse correlation between the effect of an inversion on crossing over within the tetrad heterozygous for it and its effect on crossing over in a non-homologous tetrad. The discussion presented above relative to SCHULTZ's hypothesis is also pertinent to MATHER's hypothesis and leads to the same conclusion—namely, that this hypothesis also does not explain the data.

There is one type of data involving an interchromosomal effect on crossing over which may be explained by MATHER's hypothesis. SCHULTZ (MORGAN, BRIDGES, and SCHULTZ 1935) and STEINBERG coincidentally (see introduction STEINBERG 1941) found that crossing over in the autosomes was greatly increased when measured in the exceptional offspring of XXY ♀ ♀ (that is, daughters arising from XX eggs and sons from no-X eggs). BRIDGES (1916) has shown that the X chromosomes in XX eggs are non-crossover chromosomes. Hence the increase in crossing over observed in the autosomes of daughters arising from XX eggs and sons arising from no-X eggs is associated with the absence of crossing over in the X chromosomes. While this phenomenon may be explained on the basis of MATHER's hypothesis, the explanation is not the only one which fits the data and need not be the correct one. It is possible nevertheless that the increase in crossing over observed in the autosomes of the exceptional offspring of XXY ♀ ♀ is due to a different underlying mechanism from that concerned with the increase in the crossing over arising in the presence of heterozygous X chromosome inversions, since the latter increase is not necessarily associated with a decrease in crossing over in the X chromosomes.

STEINBERG and WHITE's (1939) hypothesis requires that no relationship exist between the length or position of the inversion relative to the chromosome ends and the interchromosomal effect of the inversion on crossing over. They suggested that the interchromosomal effect of inversions on crossing over was

due to an unspecified physiological effect caused by the inversion. It was pointed out that many inversions are known to cause physiological effects.

It is now clear that most of the physiological (mutational) effects associated with inversions are due to position effects. Furthermore, neither the position of the inversion relative to the chromosome ends, nor the size of the inversion are related as such to the position effect resulting from the inversion. It has been demonstrated above that neither of these two factors are related to the interchromosomal effect of inversions on crossing over. Earlier experiments (see SCHULTZ in MORGAN, BRIDGES, and SCHULTZ 1932, 1933, 1935; STEINBERG 1937, *et al.*) have shown that the increase in crossing over is affected in all chromosomes of the nucleus. STEINBERG (1937) showed that the relative magnitude of the effects exhibited by the various chromosomes cannot be explained as a simple function of the chromosome lengths. He showed that the magnitude of the increase in crossing over per unit map length of a given chromosome was a function of the total chromatin of the cell contained in that chromosome. For these reasons the present authors postulate that the interchromosomal effect of inversions on crossing over is the result of a position effect.

Position effects may be classified into two groups (with some possible exceptions which will be discussed below): (a) those which arise as the result of the transference of a locus which ordinarily lies close to the heterochromatic region to a euchromatic region (for example the cubitus interruptus and light loci) and (b) those which arise as the result of the transference of a locus which ordinarily is situated in a euchromatic region to a heterochromatic region (for example, the white and brown loci). A group of possible exceptions is constituted of those position effects which arise as a result of translocations or inversions in which both breaks occur in euchromatic regions. However, PROKOFYEVA (1939) and KAUFMANN (1939) have shown that interstitial heterochromatic regions exist within the euchromatic regions of the X chromosome. It is conceivable that the latter group of position effects simply involve transfers from euchromatic regions to interstitial heterochromatic regions and vice versa and hence are not exceptions at all. It is our belief that the position effect leading to a change in crossing over arises exactly as do all other position effects and is subject to the same influences that they are. If this is so, the presence of a Y chromosome should enhance or decrease the interchromosomal effect of inversions on crossing over just as it enhances the position effect of some loci (the cubitus interruptus group) and decreases that of others (the brown group). Furthermore some translocations should show an interchromosomal effect on crossing over (for example, a II–III translocation may effect crossing over in the first chromosome, etc.), and others should not, depending upon the regions involved.

No predictions can be made with regard to the role of homozygous inversions on crossing over in non-homologous chromosomes, since it has been shown that some position effects have no expression in the homozygous condition (cubitus interruptus, etc.), while others do (some mottled whites, etc.); nevertheless

such experiments are worth doing, since they may contribute new facts to help our understanding of the problem.

Despite the many theories evolved to explain the mechanism of crossing over, we are still far from an understanding of its basic nature. One difficulty lies in the fact that the present techniques used to study crossing over have about reached the limit of their usefulness without giving us the information needed to solve the problem. It is to be hoped that further studies of the relationship postulated in this paper between the interchromosomal effect of inversions on crossing over and the position effect phenomenon will lead to a further insight into the problem of crossing over.

SUMMARY

The effects of 12 different X chromosome inversions (table 1) on crossing over in the third chromosome were measured.

Eight of the inversions caused an increase in crossing over in the third chromosome, while the remaining four had no effect on crossing over in the third chromosome (tables 7 and 9).

Of those inversions which caused an increase in crossing over in the third chromosome, all except the y^4 inversion did so without reducing the interference value. In the presence of the y^4 inversion interference is greatly reduced. These observations were contrasted with those of the Senior author on the effect of autosomal inversions on crossing over in the X chromosome in which it was found that a marked decrease in interference occurred in each of the three test crosses.

There is no relation between the size of the inversion, nor its position relative to the chromosome ends, nor its effect on crossing over in the X chromosome and the magnitude of its effect on crossing over in the third chromosome.

The data were examined in the light of the three hypotheses (SCHULTZ, MATHER, and STEINBERG and WHITE) which had been advanced to explain the interchromosomal effect of inversions on crossing over; only that of STEINBERG and WHITE was found adequate. This hypothesis, which in its original form ascribed the interchromosomal effect of inversions on crossing over to an unspecified physiological (mutational) effect of inversions, has been modified to state that the interchromosomal effect of inversions is due to a position effect.

TABLE 10

Number and types of offspring derived from each of the various crosses listed below.

REGION	CONTROL (1)		bb^{D1}		*dl-49*		*ClB*		sc^{7}		sc^{8}		y^{4}		*AM*		sc^{4}	
	ru	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*
0	206	359	282	318	143	167	210	243	232	335	145	177	221	274	140	186	182	281
1	134	90	171	135	80	66	108	101	167	101	76	47	103	100	85	57	123	94
2	101	93	116	96	67	60	87	80	168	118	85	66	108	85	68	53	78	104
3	6	1	5	6	3	2	2	1	7	3	2	2	5	7	0	4	0	2
4	14	20	28	37	20	20	23	35	31	41	24	25	62	46	8	22	18	26
5	44	61	57	54	36	45	60	59	85	91	28	55	84	89	31	46	36	51
6	58	54	36	45	28	37	43	43	56	83	36	35	44	43	26	24	41	49
7	188	243	143	149	117	116	116	135	167	240	76	115	159	189	80	105	123	160
1.2	14	14	23	28	8	16	20	11	18	31	19	14	24	26	7	6	8	13
1.3	0	2	2	3	0	0	1	0	3	1	0	0	0	2	1	1	0	0
1.4	9	6	17	21	7	7	9	9	12	10	5	14	18	13	3	5	2	9
1.5	25	20	23	27	13	14	31	21	38	25	17	18	44	18	17	18	16	22
1.6	21	13	16	19	16	13	17	15	28	26	15	6	31	19	16	15	22	24
1.7	83	58	86	57	54	44	67	56	142	78	42	38	67	57	63	46	55	62
2.3	0	0	1	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0
2.4	1	5	10	10	2	3	3	3	13	11	8	6	12	16	3	4	8	8
2.5	20	19	17	14	15	16	30	23	36	27	14	19	33	37	13	18	21	19
2.6	22	11	18	18	14	10	27	12	31	31	13	11	23	22	8	12	17	16
2.7	61	57	64	50	46	38	65	49	100	70	51	35	73	48	40	33	59	43
3.4	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0	0	0	0
3.5	3	0	0	0	0	0	1	0	0	0	1	2	1	1	0	0	0	0
3.6	1	0	0	1	0	0	0	1	2	0	1	0	2	1	0	1	1	1
3.7	0	0	2	1	0	1	1	0	1	3	2	1	1	4	1	0	1	2
4.5	4	2	1	4	7	3	0	5	7	6	7	8	17	20	0	3	5	7
4.6	2	4	4	3	1	4	2	3	2	4	0	5	7	8	0	2	4	2
4.7	12	15	19	15	10	9	19	13	22	28	16	17	27	30	4	7	10	13
5.6	0	3	1	0	0	4	1	0	2	3	2	2	3	7	2	1	1	3
5.7	23	24	27	21	24	34	30	42	22	53	26	21	45	38	10	21	14	29
6.7	9	13	16	10	12	5	11	12	20	22	9	10	12	12	4	10	5	14
1.2.3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
1.2.4	0	1	1	1	0	1	0	2	2	0	0	0	3	5	0	0	0	1
1.2.5	5	5	3	2	1	5	4	1	8	4	7	3	3	2	0	1	0	2
1.2.6	2	5	4	2	1	1	4	3	3	5	3	3	3	5	2	2	2	1
1.2.7	10	13	13	6	10	13	9	7	16	24	9	14	6	12	6	4	7	5
1.3.4	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
1.3.5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

| | | |

TABLE 10—*Continued*

REGION	CONTROL (1)		bb^{D5}		*dl-49*		*ClB*		sc^{7}		sc^{8}		y^{4}		*AM*		sc^{4}	
	ru	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*	*ru*	NON-*ru*
2.4.5.6	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
2.4.5.7	1	0	1	2	0	2	1	0	0	0	0	0	0	0	0	0	0	0
2.4.6.7	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0	1
2.5.6.7	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0
3.4.5.7	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
3.4.6.7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.5.6.7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
1.2.4.6.7	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0
1.4.5.6.7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
2.3.5.6.7	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.4.5.6.7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Totals	1150	1269	1271	1249	779	806	1071	1045	1547	1569	794	816	1356	1355	668	740	908	1098
Grand Totals	2419		2520		1585		2116		3116		1610		2711		1408		2006	

TABLE 11

Raw data derived from the last four inversions tested and their control (data of the two sexes is combined).

INVERSION TESTED	CROSSOVER REGIONS																TOTAL
	0		1		2		3		1.2		1.3		2.3		1.2.3		
	h	NON-*h*	*h*	NON-*h*	*h*	NON-*h*	*h*	NON-*h*	*h*	NON-*h*	*h*	NON-*h*	*h*	NON-*h*	*h*	NON-*h*	
Control (2)	1126	1385	356	327	90	94	179	199	16	17	62	51	7	7	1	1	3918
AB	476	711	229	190	76	74	110	136	17	20	44	35	13	10	2	1	2144
A 99b	526	807	196	166	53	53	95	138	12	12	37	30	8	9	0	3	2145
sc^{260-14}	606	886	240	243	60	70	122	147	17	16	32	48	9	6	1	2	2505
B^{252-47}	345	981	222	125	62	63	81	159	8	7	31	25	6	6	1	2	2124

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THE MELANINS. I. STUDIES OF THE HAIR PIGMENTS OF THE GUINEA PIG[1]

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INTRODUCTION

ALTHOUGH the chemical and physical characteristics of the melanin pigments have been widely studied, the differences which give rise to the many colors and intensities have not as yet been satisfactorily determined. Geneticists have been handicapped by this lack of knowledge when they attempted to provide a logical chemical background for known hereditary relationships from which to obtain important knowledge of genic action. For example, the chemical relationships between the melanins of different colors are not known, and it is not certain that any consistent chemical difference distinguishes them. It will be the purpose of the present work to show that the melanins from guinea pig hair may be chemically distinguished by means of the spectrophotometric technique.

RAPER (1927) has investigated the tyrosinase-tyrosine reaction, long considered the most likely source of melanin, and has found that black melanin is formed by the oxidation and increase in molecular size of certain products from the reddish-colored quinone of 5, 6-dihydroxydihydroindole-2-carboxylic acid. HAEHN (1921) also considers that an increase in particle size yields the black melanin, with intermediate colors—brownish red, violet brown, dark brown, etc., due to intermediate particle sizes. All these colors are actually found in the hair of mammalia but have not been proven to be related as have the differently colored products of the tyrosine-tyrosinase reaction. However, all the pigments in this study of guinea pig hair are referred to as melanins. That differences in particle size of a single chemical substance may be responsible for this range in color often has been suggested (JANKOWSKY 1935).

Probably the earliest spectrophotometric work with melanins was that of GALLERANI (1923) and others in Italy. They attempted to show that melanins are derived from pyrrolic compounds and not from tyrosine. DANIEL (1938) investigated the absorption curves of black, several forms of dilute black, and chocolate mouse melanins and demonstrated that these are similar except for concentration differences. Moreover, she found that the curves she obtained closely resembled that of black horse hair melanin, as reported by ZWICKY and ALMASY (1935). She concluded that the pigments are probably chemically identical. ZWICKY and ALMASY (1935) also determined the curve for red horse hair melanin and declared that the red and black pigments are spectroscopically indistinguishable. From examination of their data, however, it does not

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seem possible to make the two curves coincide by multiplying one or the other by any concentration factor, as should be the case if the two curves were given by the same substance. ARNOW (1938) has previously expressed the opinion that the red and black curves of ZWICKY and ALMASY appear dissimilar. Statistical methods for comparing the curves given by solutions of the different melanins have not been used by any of the above authors.

METHODS

Solutions for spectrophotometric comparisons were made up in the following manner. After defatting by extraction with CCl_4 for two or more hours in a Soxhlet apparatus, the hair (0.125 gm sample) was boiled in 1 percent NaOH for approximately two hours. Water was added as needed to keep the volume approximately constant. After cooling, the solutions were filtered, made to 100 cc volume, and a portion used in a Bausch and Lomb spectrophotometer to determine the absorption curve. The setting of the spectrophotometer was checked to the sodium D lines during the course of the experiments. Absorption was determined at four wave lengths, since it was found that these sufficiently described the curves between 470 mμ and 600 mμ. The averages of the readings (usually ten in number) at each point, and often made by two individuals independently, determined the curve.

During filtering, some pigment was usually retained on the filter paper. The amount so held apparently depended on variations in heating, the amount of adsorption by the keratin residue, and the kind of pigment involved. Black melanin solutions left the filter paper quite black, while red melanin was only slightly retained under similar conditions. DANIEL (1938) has noted that the shape of the curves is not affected by this filtering out of part of the pigment, since all the pigment of a given sample when dissolved gave the same curve as the first fraction. The curves given by solutions of black melanin from guinea pig hair, however, were altered by five hours boiling time; therefore, the period of extraction with the boiling alkali was limited to two hours or less.

TABLE 1

Description and genetic composition of melanins.

NUMBER	GUINEA PIG NO.	DESCRIPTION	GENETIC COMPOSITION
1	W908.4	intense black *E*	*P Sm Ee C aaBb didi*
2	W23.1	intense black e^p	*P Sm* e^p *C aaBB didi*
3	V200.1	c^r intense black	*P Sm* e^p $c^r c^r$ *aaB*
4	V876.4	c^r dilute black	*P Sm* $e^p e$ $c^r c^r$ *aaBB*
5	X91.1	intense chocolate	*P Smsm E-C-aabb didi*
6	S756.2	cherry red *bb*	*P Sm eeC bb*
7	U218.4	cherry red *B*	*P Sm eeC Bb*
8	W311.1	albino	$c^a c^a$
9	—	dopa-melanin	—
10	—	intense black human hair (Caucasian)	—

The description by which a particular melanin is designated is presented in column three, table 1; in column four is shown the known genetic composition. The first two melanins are both black, but they differ in that the second comes from an animal having red spots (due to e^p) and the first from one that is entirely black (due to *E*). One would expect these two black melanins to behave similarly when compared spectrophotometrically. Most black and chocolate guinea pigs have hair less intensely pigmented at the base than distally; however, the *di* gene carried by melanins one, two, and five causes the hair to be almost uniformly pigmented. Melanins three and four differ from one and two in carrying gene c^r in place of *C*, and for this reason such hair appears less intensely black. Of the two, number four is definitely more dilute in appearance than three, although the inheritance of the genes responsible for this difference has not as yet been determined. The black guinea pig melanins might be ranged, then, in order of decreasing intensity of the blackness of the hair: 1 and $2 > 3 > 4$. Melanin five corresponds to one and two in intensity, but since it carries gene *b* for chocolate instead of *B* for black, it is intense chocolate in color. Numbers six and seven also differ only in that one carries *b*, the other *B*, but since these animals are also *ee*, which produces red, the chocolate pigment in number six and the black pigment in number seven are restricted to the skin of the ears, eyelids, nose, etc., leaving the hair entirely red. Cherry red is a term which distinguishes the dark red characterizing these animals and others of "show" type from the lighter red ordinarily found in laboratory stock. The albino, number eight, probably contains no melanin and was included in order to have a check on the effect of keratin degradation products. Dopa-melanin, number nine, is the designation given the solution resulting when 1 gm dopa (3, 4-dihydroxyphenylalanine) in 1 liter of water stood for approximately a year stoppered and in a paper cover, during which time it had oxidized to a very dense black solution with the formation of a small precipitate. The tenth melanin was obtained by dissolving samples of very intense black Caucasian human hair.

RESULTS AND DISCUSSION

DANIEL (1938) and SPIEGEL-ADOLF (1937) have observed that when the logarithm of optical density of melanin solutions is plotted against wave length, apparently linear curves result (between 250 mμ and 670 mμ). In the present experiments this method of graphing was adopted, but in addition, statistical methods of comparing the curves were used in order to obtain a more accurate measure of slight curve differences. A straight line may be fitted to the experimental points by the method of least squares, and this method gives a value, the regression coefficient, which expresses mathematically the slope of the calculated best line. For each sample of melanin, the logarithms of the optical densities at the four wave lengths were used and the regression coefficient calculated. The average regression coefficient and the number of samples for each melanin are given in table 2.

Curves calculated by the above method have been plotted in figure 1 for representative samples of several of the melanins. In order to compare these

TABLE 2

Average regression coefficients of log optical density against wave length for alkaline solutions of various melanins.

NO.	DESCRIPTION	SOLUTIONS PREPARED BY 2 HOURS' BOILING		SOLUTIONS PREPARED BY 2 WEEKS' EXTRACTION OF HAIR AT ROOM TEMPERATURE	
		NO. OF SAMPLES	AV. REGRESSION COEFFICIENT	NO. OF SAMPLES	REGRESSION COEFFICIENT
1	intense black *E*	10	−.020	1	−.042
2	intense black e^p	3	−.020	1	−.040
3	c^r intense black	6	−.025	1	−.040
4	c^r dilute black	2	−.033	1	−.040
5	intense chocolate	2	−.029		
6	cherry red *bb*	6	−.060	1	−.069
7	cherry red *B*	2	−.068		
8	albino	4	−.047		
9	dopa melanin	3	−.019		
10	intense black human hair	2	−.016		

and DANIEL's data, her curve for heterozygous Chinchilla mouse is also given. Genetically, this melanin of hers corresponds to c^r black guinea pig melanin and would be expected to have a similar spectrophotometric curve.

Regression equations for the curves of figure 1 are as follows:

intense black (*E*)	E = 1.018 − .019X
c^r intense black	E = 1.175 − .025X
cherry red (*bb*)	E = 2.734 − .059X
albino	E = 1.284 − .048X

The regression coefficient is the number preceding the X and has been calculated as the change in logarithm of optical density per change of 10 mμ in wave length. The negative sign indicates the inverse relationship. The curves appear linear in the range investigated, and there is reason to believe that this relationship holds for melanins free from protein in the ultra violet (SPIEGEL-ADOLF 1937).

The two black guinea pig melanin curves (*C* and c^r) and the black (Chinchilla) mouse melanin curve appear to have approximately the same slope in figure 1. Since that of the latter is about −.030, two curves whose regression coefficients differ as much as .011 are not readily observed to be different in slope by inspection of such a figure. Such a degree of similarity between two curves has been taken by previous workers to indicate that the melanins involved are spectrophotometrically indistinguishable and probably chemically alike. Within the above limits, it is evident from figure 1 that black guinea pig melanins give curves like black melanins from mouse hair and from horse hair. However, as will be shown later, the slight difference between these two black guinea pig curves, .005 in amount, is statistically significant. It follows

that a statistical treatment is more likely than visual inspection to reflect the differences which do occur. The curve for red guinea pig melanin, however, may readily be distinguished statistically from those of any of the black melanins. It is evident even from inspection that no shifting of the red curve

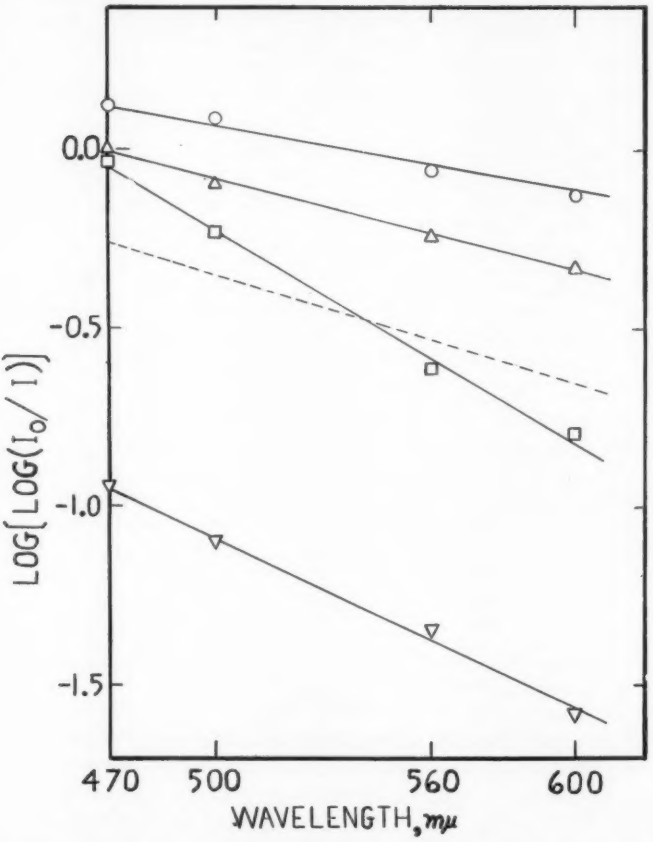

FIGURE 1.—Absorption curves of alkaline melanin solutions, log optical density (log [log (I_0/I)]) plotted against wave length. Guinea pig melanins: [o, intense black (*E*)], (Δ, c^r intense black), [□, cherry red (*bb*)], (▽, albino), [- - - - - -, heterozygous chinchilla mouse melanin, after DANIEL (1938)].

up and down by use of concentration factors (which does not change the slope of the curve) can make it coincide with any of the black curves. ZWICKY and ALMASY'S statement that black and red melanins may not be distinguished spectroscopically does not hold for guinea pig melanins. This evidence also would not support an explanation that the fundamental difference between

hairs of different colors depends entirely on the amount of pigment present. RUSSELL'S results (1939) seem to lead to the same conclusion, for she found that intense red guinea pig hair required more $KMnO_4$ to oxidize the pigment than did an equal weight of the extremely dilute black and chocolate hairs. EINSELE (1937) has suggested that qualitative differences may exist even between black and chocolate melanins, for equal weights of pigment from mice of different genotypes dissolved at the same concentration gave different color intensities in a colorimeter. In our experiment the regression coefficient for intense chocolate guinea pig melanin falls between those of the two c^r black melanins. Consequently, some physical differences not reflected by the spectrophotometric method must differentiate chocolate from black melanin.

The other curve in figure 1, albino, has been used by ZWICKY and ALMASY (1935) as a correction for the absorption of keratin degradation products. They subtracted this correction from the pigment curves. This procedure has not been followed here because the results are only slightly changed, and the albino curve has been found to be subject to considerable experimental error.

The extreme range between regression coefficients for the same melanin was .013 and occurred between two samples of cherry red *bb*. Since the other five samples were grouped around −.062, it is not unlikely that the low sample (−.050) was in error; nevertheless, all six are averaged together to give the regression coefficient −.060 for cherry red *bb* melanin. The ten samples of intense black (*E*) melanin ranged from −.017 to −.024, the next largest spread in values for a given melanin. These averaged −.020, and the variation can be attributed to experimental error. When the regression coefficients for c^r intense black and cherry red *bb* were compared statistically, it was found that the probability of their being random measurements of the same population regression coefficient is far less than one in a thousand ($t = 25$, 0.1 percent level $= 3.5$). Statistically highly significant differences may also be found among the regression coefficients of the black melanins (for intense black (*E*) and c^r intense black, $t = 9$, 1 percent level $= 2.7$). This may confirm EINSELE'S evidence for qualitative differences among different black and chocolate melanins, but the number of samples in all cases is small. The c^r dilute black curve is significantly different from the intense black (*E*) curve and, moreover, bears the same relation to it that the dilute black curve of DANIEL (1938) bears to her intense black mouse melanin curve. As DANIEL stated, the differences between the two appear small when the plotted curves are examined. However, the use of regression coefficients may indicate whether such small differences are consistent or without significance. DANIEL has further suggested that the curves may represent scattering and not true absorption. If this is the case, further study of melanin curves might lead to information of the relative particle sizes involved. Even if melanin solutions are not true solutions, conclusions from spectrometric observations are probably valid, since the Lambert-Beer Law has been shown to be generally true for hydrophobic colloids and in particular for solutions of melanins (See SPIEGEL-ADOLF 1937).

ZWICKY and ALMASY (1935) state that their solutions, obtained by extrac-

tion of the hair for two weeks at room temperature, did not alter in optical properties on long standing. Others found, however, that solutions prepared by boiling were apparently bleached by exposure to light or ultra-violet light or on further boiling (DANIEL 1938; EINSELE 1937). DANIEL (1938) has presented curves from samples of hair boiled 30 minutes and 165 minutes to show that the slope is unchanged although the apparent concentration decreases. In the present experiments not only were changes found in the apparent concentration of pigment as measured by the height of the spectrophotometric curve, but also the slope of the curve was altered on standing.

Table 3 illustrates these changes for intense black (*E*) melanin. The first line records the average optical density of two samples at 470 mμ at the time intervals indicated, while the second line gives the average regression coefficient for the two curves. The values at 120 days are from a different sample of the same melanin. Red melanin solutions in general changed much less rapidly but must be further studied before drawing definite conclusions. The average regression coefficient of two samples of red melanin changed from −.062 to −.069 after 18 days.

TABLE 3

Changes in optical density and regression coefficient of intense black (E) melanin solutions with time in 1 percent NaOH.

DAYS	0	1	2	4	7	11	18	120*
Optical density at 470 mμ	1.53	1.23	1.18	1.02	0.91	0.82	0.74	0.23
Regression coefficient	−.022	−.025	−.028	−.032	−.033	−.036	−.039	−.048

* Readings at 120 days made on a different sample of the same melanin.

Using ZWICKY and ALMASY's method of extracting the hair two weeks with alkali, solutions were prepared which gave the regression coefficients in the last column of table 2. These values check quite well with those of table 3 for between 11 and 18 days; so it may be concluded that the changes which take place in the boiled solutions on standing also occur when ZWICKY and ALMASY's method is employed. The greater similarity of the curves after 14 days may explain in part why ZWICKY and ALMASY decided the curves for red and black melanin are alike.

The change in slope of the curve of the black melanin solution on standing obviously brings it nearer that of the red melanin curve. ARNOW (1938) oxidized a solution of dopa melanin in air to a reddish solution having the same spectrophotometric curve as an extract of red human hair. Thus he concluded that red melanin is an oxidation product of black melanin. A comparison of ARNOW's red melanin curves with those of red guinea pig melanin shows that the latter are definitely more steep. The former would have a regression coefficient of approximately −.04 while that of red guinea pig melanin is −.06. A sample of dopa melanin oxidized in this laboratory by bubbling air through it for two days in 1 percent NaOH changed its regression coefficients from

−.021 to −.039, but seemed to reach an equilibrium at that point. Treatment of this product with H_2O_2 produced a yellow solution with a regression coefficient of −.062. ARNOW's conclusion that red melanin is an oxidation product of black melanin apparently holds for guinea pig melanins, although red guinea pig melanin seems to have a definitely steeper curve than other red melanins yet reported.

A comprehensive theory of the processes involved in the melanin pigmentation of mammals has been proposed by WRIGHT (1917, 1927) and related to the known color genes in guinea pigs. His Substance I is determined by the *C* locus and is necessary for any pigment production. If Substance I is present in a concentration corresponding to c^r or a higher allele, black or chocolate pigment may be formed if Substance II determined by gene *E* is present; or red pigment may be formed if Substance I is present in a concentration corresponding to c^d or a higher allele, and Substance II is absent. The effects of other genes and conditions are also included in WRIGHT's hypothesis. Considerable investigation will be required to determine the precise effect of each gene in definite physiochemical terms, but some conclusions may be drawn from the present experiments.

If we accept the hypothesis that red melanin apparently differs chemically from black by being more highly oxidized, and since it differs genetically by having the constitution *ee* instead of *E*, it seems clear that the *E* gene determines the presence (1) of a less efficient oxidizer than *ee*, or (2) of an inhibitor of the oxidation process, or (3) of some other condition unfavorable to the oxidation of melanogen past the black or chocolate stage. Whichever alternative is true is represented in WRIGHT's scheme by Substance II. Gene *E* may then as well produce the absence of some agent in the oxidation process (or a lower concentration than *ee*) as the presence of a "Substance II."

The *B* gene produces an effect (in the hair) only in the presence of both Substances I and II, in which case it produces black pigment in contrast to true chocolate pigment produced by *bb*. The nature of the difference in action of *B* and *b* is not known, but the present work shows it is not so much a chemical difference, which should be detectable with the spectrophotometer, as a physical state, such as density or particle size of the pigments involved.

BOGART and IBSEN (1937) and IBSEN and BOGART (1939) assumed that when black hair was bleached with H_2O_2, black melanin became colorless and allowed red pigment to show. Since the present results have shown that the oxidation of black melanin in solution either by air by by H_2O_2 produces a reddish solution, another possible interpretation is that treatment of black hair with H_2O_2 changes the black pigment to red.

SUMMARY

The use of regression coefficients for log optical density plotted against wave length is proposed as a means of comparing the absorption curves of melanin pigments. By this method red and black guinea pig melanins have been shown to be easily distinguishable.

Some evidence is found that qualitative differences may exist between dif-

ferent black guinea pig melanins. However, their curves are similar to those of black melanins from mice and from horses, and also to that of dopa melanin.

Solutions of black melanin prepared by boiling hair two hours in 1 percent NaOH do not give the same curve after standing 24 hours; the resulting curve decreases in height and its slope shifts toward that characteristic of red melanin. Air oxidation is presumed to be the cause.

The regression coefficient for intense chocolate melanin falls within the range of those for different dilute black melanins; hence it is concluded that chocolate and black melanins are chemically alike and that the *B* locus probably determines some difference in physical state of the pigment present.

Additional evidence that red melanin is an oxidation product of black melanin is presented, and this difference is related to the *E* locus (in guinea pigs) in terms of WRIGHT's hypothesis regarding melanin pigmentation.

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